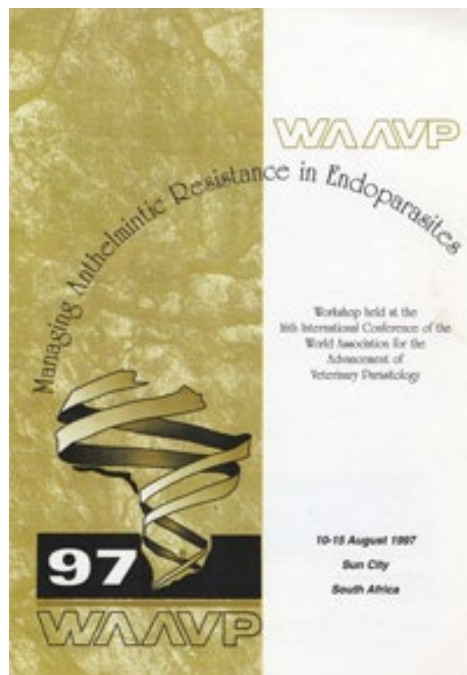


The 16th International Conference of the
**WORLD ASSOCIATION FOR THE
ADVANCEMENT OF VETERINARY
PARASITOLOGY**

“Managing Anthelmintic Resistance in
Endoparasites”

August 10-15, 1997

SUN CITY, SOUTH AFRICA



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Managing Anthelmintic Resistance in Endoparasites

Workshop held at the
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Managing Anthelmintic Resistance in Endoparasites

Editors:

J. A. van Wyk
P. C. van Schalkwyk

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Preface

The excellent anthelmintics developed over the past three decades carried with them the seeds of their own destruction. Such outstanding results were obtained in controlling most helminths that these products were used, and often over-used, to the virtual exclusion of any other control methods. Pharmaceutical organisations quite understandably took the lead in parasite research and the funding of research in other directions was all too often deemed unnecessary by uninformed but powerful outsiders. The development of anthelmintic resistance by a range of endoparasites was an inevitable by-product of this over-reliance on anthelmintics to control worms, and it has been made clear to just about every person in the field of animal production that anthelmintic resistance is a major and growing problem which threatens the very viability of certain types of stock farming unless we change our approach.

For a while it seemed that research in other directions had faltered, but groups around the world have taken up the challenge, resulting in a number of novel and promising avenues for research being actively followed.

Being in Africa with its rich variety of wildlife and their diversity of parasites, gives us the advantage of seeing complex ecological equilibrium in operation at first hand. This leads us to the conclusion that in the long run, we will have to manage parasites, their hosts and environment as a complex but single system in which adaptation and equilibrium must be the long-term goal.

These Proceedings bring together invited review papers by active scientists from countries around the world. Each review

presents challenging ideas of the ways in which the spectre of widespread and severe anthelmintic resistance can be overcome. While there are several warnings on the continued and escalating spread of resistance in worms, there are several promising ways outlined by which these effects can be minimised.

The chemotherapeutic agents which are available can be better utilised, and their effective lives prolonged, by a range of methods and measures outlined in the papers, such as the use of proper treatment programmes, more effective methods of drenching, prolonged release formulations, and optimised pharmacokinetics.

The way in which dissemination of resistant strains may occur, is dealt with, while an idea on how resistance can be reversed (dilution by substitution) is put forward. Another paper deals with the importance of ecologically critical treatment times in promoting resistance, and suggests that deliberately withholding treatment in a proportion of the flock will significantly slow the increase in genetically resistant worm strains. The genetics of resistance and resilience are outlined in a further paper, and the authors suggest that the simultaneous application of selection strategies for both attributes would be the preferred method to breed adapted animals.

Biological control options are reviewed with particular emphasis on predacious fungi and practical delivery systems for spores. Bacterial, vertebrate and invertebrate candidates are also evaluated. Vaccines have been researched for many years and there now seems to be a potentially viable system under development, based on hidden (gut) antigens for haematophagous

parasites like *Haemonchus*.

The reviews are authoritative and challenging. Together they demonstrate that although the situation regarding anthelmintic resistance is very serious, there are a number of practical possibilities for effectively managing verminosis in spite of this problem. These research directions

must be actively pursued and deserve the full support of the animal production industries, as well as governments.

Readers will find these Proceedings to be a very good synopsis of the present state of knowledge and activity on ways to manage worm resistance, and the directions in which future investigations will probably proceed.

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BIOLOGICAL CONTROL OF HELMINTHS

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ABSTRACT

Novel approaches to the control of nematode parasites in small ruminants are now becoming an urgent priority, largely because of the rapidly escalating problem of anthelmintic resistance in the major sheep raising countries of the world. Research on the biological control of parasites, directed at the free-living stages on pasture, has made rapid progress in recent years. Work in this area has focused on exploiting the nematode destroying properties of certain micro fungi, which have the ability to either survive gut passage in ruminants and then germinate and spread rapidly in freshly deposited dung, or of fungi that rapidly colonize fresh dung once deposited on pasture. These have been shown to capture infective larvae before they migrate from dung, resulting in not only parasitological, but also production benefits of grazing livestock.

However, the pursuit of candidates for the biological control of nematode parasites should not involve just the micro fungi. There is a large range of other organisms, which can have direct, or indirect, effects on the number of free-living stages of parasites. These deserve closer research attention.

With the current rate of progress, and the level of commercial interest in the biological approach to nematode parasite control, it is not unreasonable to expect that biological control products will become available in the near future — certainly before the turn of the century. These will become valuable weapons in the non-chemotherapeutic arsenal of nematode control agents, which need to be used by livestock producers to ensure that effective, but at the same time sustainable, parasite control can be reasonably assured for the future.

INTRODUCTION

Biological control for any target pest organism is aimed at exploiting its natural enemies to reduce the number of the pests in the environment to a level less than what would have occurred in the absence of the biological control organism(s). So is it with helminth parasites. For more than 50

years, natural enemies to nematode parasites have been reported in the literature and some attempts had been made to control worm parasites of man and livestock by these organisms (for reviews see Grønvold, Wolstrup, Nansen, Henriksen, Larsen & Bresciani 1993; Waller & Larsen 1993). However, it was not before the detailed and systematic investigation by the workers in Denmark, pioneered by Grønvold in the early 1980s, that the concept of biological control of nematodes in livestock was to be taken seriously. Since this time, progress by any assessment criterion has been extremely successful and commercial biological control products and/or methods to facilitate the action of these organisms should be available before the turn of the century.

Biological Control Agents of Helminths

Vertebrates

In the broadest sense, any means by which animals are separated from their faeces (i.e. free-living stages of parasites) constitutes a form of biological control. Thus it could be argued that man's manipulation of livestock movement and numbers could be classified as a form of biological control. Evasive parasite control brought about by movement of animals to avoid the peak periods of larval pick-up from pasture, is an excellent, albeit indirect form of biological control. Likewise selling young, susceptible livestock to the slaughterhouse deprives parasites of susceptible hosts in which they may readily complete their life cycle. Without such intervention by man, parasite numbers would be higher in set-stocked animals. On the other hand, it could be argued that man's influence in the domestication and intensification of livestock has tipped the balance in favour of the parasite in the first instance. One activity of man which could more justifiably be argued to be a form of biological control, albeit mostly incidental, is the practice of collecting dung to use as fuel, building material etc, thus breaking the life cycle of parasites. However, in regions of the world where this is practised it is generally restricted to bovine dung, weather conditions are often hot and dry, and malnutrition rather than parasitism is the major cause of loss in livestock productivity.

Certain birds seek out coprophilous arthropods as a component in their diet. This food source can be of utmost importance for juveniles of some species of birds, which lack the food-gathering skills of adults and have thus become heavily dependent on dung as a food source (McKracken 1993). In seeking their prey, these birds usually break open and widely scatter large deposits of cattle and horse faeces, thus allowing much quicker desiccation of the faecal material than would otherwise occur in the intact pats. Dung pats and piles have been shown to provide an important buffering capacity against both extremes of temperature and moisture, in this way enhancing the development and survival of parasite free-living stages (Anderson, Donald & Waller 1983). Except in very isolated situations as mentioned by McKracken (1993), the number of birds involved in this activity is unlikely to be large enough to have a measurable and consistent effect on parasite numbers on pasture.

Invertebrates

Two important groups of organisms that can have an effect on the free-living stages of parasites, are dung beetles and earthworms.

Dung beetles

Dung beetles, found throughout the world, are often capable of rapid and complete dung removal and thus are indirectly responsible for significant reductions in the number of free-living stages of parasites (for review, see Waller & Faedo 1996). However, being dependent on ideal weather conditions, such dung dispersal activity is notoriously labile, with the result that little opportunity exists to exploit these organisms in attempts to achieve cost-effective and reliable biological control of nematode parasites.

Earthworms

Earthworms take over the role of dung beetles in the cool, moist regions of the world. In northern Europe for example, earthworms play an important and often dominating role in removal of cattle dung from pastures and can be responsible for significant reduction of infective larvae on pasture (Grønvold, 1987).

Micro-arthropods

Macrochelid mites can occur in large numbers attached to dung beetles on which they are transported to fresh dung. These minute insects are carnivorous, primarily seeking out fly larvae

as prey, but in their absence will include nematodes in their diet (Waller & Faedo, 1996).

Micro-organisms

A number of micro-organisms have been shown to exploit the free-living stages of parasites as a source of food. These organisms include protozoa, predacious nematodes, viruses, bacteria and fungi (for review, see Waller & Faedo 1996). Although all are of intrinsic interest, it is from the latter two groups of organisms that breakthroughs in biological control are likely to emerge.

Bacteria

Many species of bacteria are associated with the cuticle, body cavity and gut of nematodes and some of these are pathogenic. *Bacillus penetrans* is a promising candidate for the control of parasitic nematodes of plants. It produces highly resistant spores, which attach to the cuticle and then invade the nematode host. This bacterium is highly host-specific, which is both a good and bad thing. It is good from the standpoint that only the target nematode pest will be affected, but bad insofar as the search for the specific *B. penetrans* pathogen for each of the whole range of nematode pests would be most laborious, expensive and fruitless in many cases. Another factor that is hampering the exploitation of this organism is the difficulty in culturing large quantities of *B. penetrans*, which is an absolute pre-requisite for commercialisation. Many bacteria and closely related organisms, the Actinomycetes, produce important secondary metabolites, including antibiotics, insecticides and anthelmintics. As such they should be regarded as microbial control agents, rather than true biological control agents.

Fungi

Fungi that exhibit anti-nematode properties have been known for a long time. They consist of a great variety of species which include nematode-trapping (predacious) and endoparasitic fungi, and others that either invade nematode eggs or produce metabolites toxic to nematodes (Barron 1977). The most important groups of nematophagous fungi are the first two, namely: Predacious fungi. These fungi produce specialised hyphal trapping devices, such as adhesive networks, knobs, and constricting or non-constricting rings. Fungi in this class may also produce nematode chemoattractant and/or chemotoxic substances (Waller & Faedo 1993).

Within a short period of time following capture of the nematode, the fungus penetrates the worm and destroys it.

Endoparasitic fungi invade the nematode from adhesive spores that stick on the cuticle, from spores that are ingested by the nematode, or from motile spores in water.

Fungi from these two classes are found in all environments throughout the world, but are particularly abundant in rich agricultural soils. Under laboratory conditions, where fungi are grown as a monoculture on standardised, generally nutrient-poor media and are provided with a nematode prey that cannot escape, results can be spectacularly successful. Nematodes can be totally captured and destroyed within a matter of hours. However, this type of work provides little relevant information as to how these fungi would perform as practical biological control agents against animal parasitic nematodes under field conditions. The limitations and opportunities should be tested for parasite control associated with the livestock production systems being considered.

Methods for Selecting Fungi as Biological Control Agents

The most important principle for selecting candidate fungi as possible biological control agents is to obtain isolates from the field in the region, or country, where this work is to be performed. This is important for several reasons. Firstly, it has been observed that, following repeated passage, laboratory stocks of fungal isolates lose various attributes, which may include nematode-destroying capacity. Secondly, most countries have stringent requirements regarding the importation and field release of exotic living organisms. These two drawbacks would apply if strains of fungi with known nematophagous activity were obtained from the major fungal collections, or repositories, in Europe or North America. Fungal species that have evolved to survive under local environmental conditions would in any case be much better strains to work with, than those derived from centralised fungal collections.

The most relevant sites for sampling would be the environments where the fungi are expected to exert their effects, notably fresh faecal deposits, but animal bedding may also be appropriate in intensive animal production

systems. The reason for restricting the sampling to these sources is to save unnecessary labour at a later stage, because in almost all circumstances deployment of fungi will be in ways, which require them to survive passage through the gastro-intestinal tract of animals, without losing the ability to trap nematodes in freshly deposited faeces. Almost certainly, a large number of nematophagous fungi would be isolated from other sources such as soil, pasture, etc., but almost all would fail the most important test of gut survival, in the absence of which their isolation (and any other testing) would be wasted effort.

If animals are to be used as a stringent screening procedure, then the number of occasions on which isolations can be expected, is very low. Therefore, for a serious attempt to be made, collect a large number of small samples *per rectum*, from livestock found on a comprehensive range of farms in the region, and use isolation procedures described by Larsen, Faedo & Waller (1994). Thereafter, pen trials should be carried out to confirm the gut survival and nematophagous capabilities of the fungal strains.

Possible Fungal Methods/Products for Biological Control of Nematode Parasites

Direct Application

This could only be considered in the most intensive forms of animal production, where internal parasitism is a problem in animals that are closely confined. Such an example would be the intensive calf-rearing units in the southern islands of Japan, where *Strongyloides papillosus* can cause sudden death in massively infected animals in the hot summer months (Taira & Ura 1991). A practical solution to this problem may be the direct application of fungi to the bedding, an instance in which the fungi need not be able to survive gut passage. All that would be required, is for the fungi rapidly to colonise the bedding and sufficiently to reduce the overwhelming number of *S. papillosus* larvae to prevent the sudden death syndrome, while still allowing sufficient numbers to survive to provoke the normal, characteristically rapid development of immunity, which occurs against this parasite. Preliminary investigations in Malaysia have shown that this may be a possibility. A local isolate of the nematophagous fungus, *Arthobotrys oligospora*, eliminated *S. papillosus* from cultures when spores (conidia) were added

in small quantities (P. Chandrawathani, personal communication).

However, apart from other similar forms of highly intensive livestock production, it is beyond the bounds of reality to conceive of a practical means of applying fungal material, especially to the grazing environment, to produce reliable and substantial reduction in the free-living stages of parasites.

Supplementary Feeding

Danish workers have demonstrated that a daily supplement of barley grains supporting the growth of *Duddingtonia flagrans* will reduce parasitism and increase productivity in grazing cattle, pigs, horses and sheep (Larsen, Nansen, Grønvold, Wolstrup & Henriksen 1997). These results are especially exciting, as they demonstrate that the principle of biological control of nematode parasites using nematophagous fungi is particularly robust, being applicable across the whole spectrum of grazing livestock species. Clearly then, applying this technology to those industries where long-term daily supplementary feeding is a common management procedure, would be relatively straightforward. The major impediment would be the need to scale-up production of the fungal grain supplementary feed to satisfy the commercial requirements for biological control of nematode parasites.

Another exciting possibility comes from recent work in Brazil, where the endoparasitic fungus, *Harposporium anguillulae*, has been shown to profoundly reduce the number of infective larvae that emerge from faecal cultures (Charles, Roque & Santos de P 1996). This is the first investigation for this class of fungi, which are nematode invaders (endoparasitic) rather than trappers (predacious). These workers report that this fungus is very common inhabitant of cattle dung, being found in abundance after only three days following deposition on pasture. To exert its effects, this fungus needs to be ingested by feeding larvae. The "window of opportunity" for these fungi to control animal parasitic nematodes occurs quickly and is very ephemeral as the only actively feeding stages are the first and second stage larvae, which in most circumstances are found in dung for only a very short period soon after deposition. Nevertheless, this may be exploitable in the humid tropical/subtropical regions of the world. If this is the case, then the need for such fungi to survive

gut passage in livestock does not apply for them to be considered a practical proposition for biological control.

Feed Blocks

Block administration, developed mainly for mineral supplementation and to a lesser extent for anthelmintic medication, is now undergoing a resurgence of interest as a means of low-cost nutrient supplementation of livestock. These blocks can be manufactured using simple technology and those manufactured in the developing world often incorporate surplus plant by-products as the nutrient source, which may well prove to be suitable growing substrates for locally isolated strains of nematophagous fungi. A range of block formulations containing *D. flagrans* chlamydospores have been tested and the results are very encouraging (P.J. Waller & M.R. Knox, unpublished data). Fungal spores survived the manufacturing processes and resulted in significant reductions in larval numbers in faecal cultures set up during the period the sheep received the fungal infected blocks, even in sheep that consumed the blocks only modestly and irregularly. Some of these block formulations also contained viable spores for (thus had a shelf life of) at least 6 months. Fungal blocks could prove to be a particularly important control option in the humid tropics and subtropics, where tethered husbandry and night housing with stall feeding are common animal management practices and where anthelmintic resistance is a serious problem.

Controlled release devices

Intra-ruminal sustained, or controlled release devices, are a modern advance in anthelmintic medication. Although the unit costs of these devices is high, they allow great flexibility in animal management because they provide protection against parasite infection for an extended period of time. Rather than using anthelmintic compounds, devices containing fungal spores could provide this extended prophylactic effect. The objective would be to develop a device which would release sufficient spores for a period of 60 days or more, to substantially reduce the number of infective larvae migrating to pasture over that time period. These devices could be administered at epidemiologically critical times to reduce seasonal peaks in larval numbers, but would allow sufficient larvae to escape to provoke the development of naturally acquired immunity in grazing livestock.

Investigations have shown that chlamydospores of *D. flagrans* can withstand tableting pressures required for manufacturing controlled release devices, which have a good shelf life and can release optimum concentrations of spores for effective parasite control *in vivo* (P.J. Waller & K. Ellis, unpublished data). Further work is required to test the time/release profiles of fungal chlamydospores in these prototype devices and to verify the long-term *in situ* viability of spores in devices administered to livestock. Although it is premature to speculate as to whether commercially attractive, fungal controlled release devices will be developed, they have an enormous potential market as a non-chemotherapeutic, environmentally benign form of parasite control to all the grazing livestock industries throughout the world.

CONCLUSION

Modern methods for controlling worm parasites of livestock need to shift away from the reliance on anthelmintics to a more integrated approach to pest management. Biological control is a major tactic in integrated pest management (IPM) of insect pests and there are grounds for optimism that this will also apply to animal nematode infections in the near future. With the current move towards "sustainable" agriculture, biological control can be expected to play an even more substantial role in IPM of worm parasites. However, this view must be tempered with the inescapable fact that the commercial, financial and animal management dependence on anthelmintics is too great to allow for any rapid change. But the goad will be the spectre of widespread, high and escalating levels of anthelmintic resistance.

In comparison with other non-chemotherapeutic approaches to parasite control in livestock, progress in biocontrol using nematophagous fungi has been remarkable in recent years. Although commercial interest is high, there has been a general reluctance by companies involved in the anthelmintic business to invest in this research. Part of this is due to the fact that in contrast to anthelmintic discovery and development, where new drugs can be tightly protected by patents, this is not the case for naturally occurring organisms. However, livestock owners are showing an ever increasing interest worldwide in this alternative technology, which is in tune with the move towards the sustainable, ecologically and environmentally

acceptable systems of livestock management and disease control. Therefore there is a clear market for bio-control products against worm parasites of livestock.

Biological control has many obvious attractions and advantages over other non-chemotherapeutic means of worm control. For example, it will be applicable to the range of worm parasites not only within, but also between, species of livestock, which is one of the major shortcomings of the worm vaccine approach. It will provide the opportunity for livestock producers to capitalise on the increasing demands of consumers for chemical-free livestock products. Finally, it is also difficult to envisage the development of resistance mechanisms which cast a dark shadow over the future of anthelmintics.

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PRACTICAL ASPECTS OF PARASITE TREATMENT

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ABSTRACT

Extensive development of resistance to current chemical classes of broad spectrum anthelmintics poses an undeniable threat to the long term ability to control parasitic disease. To compound the severity of the situation there appears little likelihood that a new, unrelated chemical class of anthelmintic, with unique mode of action, will be available in the foreseeable future. If effective parasite treatment is to continue, then the chemical active ingredients that are currently available must be used more efficiently.

While care must be exercised to adhere to the basic elements of parasite treatment (including knowing the "resistant" status of the parasite population, correct selection of anthelmintic and integration of epidemiologically based strategic treatment programmes), there are a number of practical options that can be implemented to maximise anthelmintic activity.

Using low volume drench formulations and ensuring that the drench is administered over the tongue to minimise rumen by-pass due to oesophageal groove closure, will greatly assist deposition in the rumen. Withdrawing feed for 24h slows the flow rate of digesta from the rumen and this will prolong the availability of orally administered benzimidazole or macrocyclic lactone compounds. This is a cost-effective option that is being implemented in many sheep producing countries, in the process not only enhancing the efficacy of older compounds, but also prolonging the useful life of drugs to which resistance has not yet developed.

Repeated administration of the recommended dose of benzimidazole drugs at a 12h interval maintains exposure and significantly increases efficacy.

Following from this, the use of medicated feed supplement blocks and intraruminal controlled release capsules, that provide a continuous low level release of anthelmintic over an extended period, are highly effective in eliminating

susceptible populations as well as inhibiting the establishment of resistant nematode larvae.

The most promising opportunity for improving formulation lies in innovative delivery systems. Chemical or physical carriers including solubility defining salts and oils can modify drug pharmacokinetic behaviour and increase availability at the site(s) of parasitic infection. The use of these "intelligent" formulations for parenteral delivery provides a further opportunity for sustained availability of the anthelmintic compound, to provide immediate as well as prophylactic parasite control.

Clearly, understanding the pharmacokinetic/physiological interaction in the host will be instrumental in assisting in the development of improved treatment/formulation options using existing actives. This approach will undoubtedly form the basis of practical parasite treatment.

INTRODUCTION

Practical anthelmintic treatment of livestock can be defined as the action(s) that can be taken NOW by the livestock producer to maintain control over parasitic disease. With this in mind this presentation aims to discuss how present "conventional" modes of anthelmintic formulation and administration might be used more effectively, particularly against resistant strains of parasites. This approach will be essential, since:

- there is little likelihood of (a) new chemical(s) with unique mode(s) of action becoming available in at least the next decade, thus the compounds we presently use will continue to form the basis of parasite chemotherapy.
- resistance to the three chemical classes of broadspectrum anthelmintics (levamisole (LEV)/morantel, benzimidazole (BZ) and macrocyclic lactone (ML; ivermectin (IVM)-like) in nematodes of sheep, goats and swine (Waller 1994; Roepstorff, Bjorn & Nansen 1987) and cattle (Craig 1993), is eroding our ability to control parasitism. The lack of alternative, non-chemical methods of treat-

ment, at least in the immediate future, means that chemotherapy, in conjunction with effective grazing management systems will remain as the cornerstone of parasite treatment.

Although faced with these obstacles, it is important to remember that the majority of anthelmintics are still efficacious in most livestock, especially in cattle, where only few reports attribute drench failures to the occurrence of anthelmintic resistance (Craig 1993). The optimistic attitude is that parasite control can be maintained if technologies are adopted which maximise drug efficiency and if operational procedures which can compromise efficacy, are avoided. While repeated exposure of a parasite to anthelmintic must ultimately contribute to development of resistance, maximising the availability of the active in existing products by taking actions that **give the drug the best chance to work**, will be crucial for maintaining the effective life of these valuable resources.

THE BASICS

Basic procedures for anthelmintic use are well known to this audience, but their importance is often overlooked or even disregarded as simplistic. Nevertheless, they are a not inconsiderable component of the antiparasitic inventory and their fundamental importance to practical worm treatment cannot be overstated.

Know the status of the target.

Significant benefit can be obtained by regular assessment of drench performance by using faecal egg count reduction (FECRT), or similar tests. Combining such a test with larval cultures to identify susceptible species or emerging resistant populations determines the most appropriate anthelmintic chemical class to use on a particular farm.

Strategic treatment programmes

Strategic worm control programmes are based on a thorough understanding of parasite epidemiology in a particular region and have been well accepted by the Australian sheep farming industry. Treating animals at epidemiologically critical times in the year makes more efficient use of the anthelmintic and can dramatically reduce the frequency of drenching. Strategic treatment programmes have reduced

anthelmintic use in the Australian sheep industry from as many as 12 to only 2 or 3 times per year. While impossible to assess the extent to which these programmes have delayed the development of resistance, it is generally accepted that without them resistance in sheep nematodes would be significantly greater simply because of the increased frequency of anthelmintic exposure. Such strategic treatment programmes based on local or regional epidemiological information are used in most sheep producing countries.

What anthelmintic to use

All drench options will not be discussed here, but a few points are worthy of mention. To a large extent FECRT, drench rotation and strategically defined treatment will indicate which drench class to use, but more information is obtained if the larval development assay, originally described by Lacey, Craven, Gill & Baker (1993) and Gill, Redwin, van Wyk & Lacey (1995), is used. Marketed as "Drenchrith" in Australia, the assay, which uses worm eggs from faecal samples, defines the level of resistance to a specific anthelmintic class in a particular nematode population. The highly specific assay provides clear direction for selecting the most effective drench. At this point it should be emphasised that if FECRT or other diagnostic information indicates that control with a narrow spectrum product is appropriate, then this should be used in preference to broadspectrum compounds. For example, the strategic use of closantel to treat *Haemonchus contortus* can reduce the drenching frequency, and therefore the development of resistance to broadspectrum drugs.

Combination products are also of much benefit but their effectiveness is best realised against populations of worms in which the frequency of resistance genes is relatively low. Using epidemiological and climatic based computer simulation, Barnes & Dobson (1990) demonstrated that commencing with a resistance allele frequency of 5%, annual rotation of two anthelmintic classes will result in the initially slow, but increasing development of resistance to both compounds. After 10 years of use the gene frequency to resistance to both drugs will increase to about 50 %. However, if the two drugs are administered as a combined product, the level of resistance in the same population will be only 10 % after 10 years. Combination

products containing LEV and BZ are widely available and inclusion of naphthalophos with LEV or BZ is being considered (Cooper, Chick & Lyndal-Murphy 1997). ML combined with other chemical class anthelmintics could be useful in slowing the development of resistance to these drugs (Rolfe 1997).

One should also consider the integrity of drench formulation. While this is not of question with well known anthelmintics, some generic products sold by little known suppliers can come under suspicion. Assaying a number of commercial products sold in Kenya, Monteiro, Wanyangu, Kariuki, Bain, Jackson & McKellar (1997) demonstrated wide variation in the quantity and reproducibility of active in product batches. Concern must be placed on using little known or obscure generic products that might result in sub-therapeutic dosing and further contribution to development of resistance.

Finally, selecting and administering the correct dose rate is arguably the most important element in limiting the development of resistance, but it is probably the most neglected. Put simply, it is crucial that the animal is administered the maximum permissible dose quantity.

FORMULATION AND DELIVERY

Formulation philosophy

The pharmaceutical industry has been so focused on the need to develop unique actives or generic "look-alike" compounds, that the scope for improving existing drugs through formulation and delivery has largely escaped attention. In many cases excellent parasitocides have had their effectiveness limited or actually compromised by overlooking optimal formulation and/or presentation. The effectiveness of an existing formulation can potentially be increased in two basic ways. Firstly, and most simply, the existing preparation can be administered to the animal after the animal's behaviour has been temporarily altered to improve drug availability, for example to reduce feed intake before treatment, an option that will be discussed in detail later. Secondly, the formulation *per se* can be modified to increase availability by, for example, targeting the drug to the site of infection.

Utilising host physiology

We can utilise the host's physiological status to influence drug availability, but before doing so one must understand the mode of action and intrinsic pharmacokinetic behaviour of the anthelmintic compound. Considering the BZ and ML chemical classes, efficacy is principally dependent upon the **duration** of concentrations that are "toxic" to the target parasite (Lacey 1988), rather than the concentration alone. These "toxic" concentrations of parent or metabolite have never been specifically defined, but it could be suggested that the concentration that is midway between zero and the maximum concentration could be regarded as the minimum "toxic" level.

If the efficacy of existing, or new anthelmintic drugs is to be increased, the duration of the minimum toxic concentration (obviously within safety limits) should be extended. Conversely, anything that shortens the duration of drug availability can result in subtherapeutic exposure, so that individual parasites carrying alleles which increase tolerance to the drug survive, thereby ensuring a progressive increase in development of resistance.

Effective oral administration

The first consideration in treating a ruminant is to ensure that the maximum quantity of drench is delivered into the rumen. The BZ and ML compounds strongly associate with particulate matter in the rumen (Hennessy, Ali & Tremain 1994) and the extended residence time of this drug-digesta complex prolongs the supply of drug, as it exchanges into the digesta fluid phase before it is absorbed. If the drench is delivered into the buccal cavity, the act of drenching can stimulate closure of the oesophageal groove, so that a significant proportion of the drench bypasses the rumen in cattle (McEwan & Oakley, 1978) sheep (Prichard & Hennessy 1981; Sargison 1997) and goats (Sangster, Rickard, Hennessy, Steel & Collins 1991). Sargison (1997) followed the path of an orally administered barium contrast medium "drench" fluoroscopically and demonstrated that, while rumen bypass occurred frequently in young weaned animals, it decreased by 80 days after weaning. However, even in older animals and particularly during pregnancy, one third of treated sheep exhibited complete rumen bypass, a figure similar to that reported by Prichard & Hennessy (1981). Rumen bypass initially increases absorbed drug

concentrations but the duration of "toxic" concentrations and thus efficacy are significantly shortened.

The volume administered will also influence the pharmacokinetic behaviour of a drench. A large volume of BZ administered to the buccal cavity, has a greater tendency to bypass the rumen than a smaller, more highly concentrated drench formulation. Hennessy, Martin & Murray (1997) showed that the coefficient of variation about the mean area under the plasma oxfendazole (OFZ) concentration-with-time curve was 26 % with large volume, compared to only 14 % with small volume formulations. The higher variability with the large volume formulation was attributed to a greater incidence of rumen bypass. The larger the variability, the greater the risk of subtherapeutic drug levels occurring; exactly what we do not want.

The influence of drench volume will be reduced by always administering the drench over the tongue and as far as possible, directly into the oesophagus. Supporting this premise, Benchaoui, Futter, Holton, Gettinby & McKellar (1995) achieved bioequivalence with identical dose rates of albendazole (ABZ) when the dose was administered in 2.5, 5, 10 and 15 % w/v formulations, on condition that the drench was carefully administered into the oesophagus (Q.A. McKellar, personal communication). Clearly the optimal approach is to use small volume drench formulations administered over the tongue to direct the drug to the oesophagus.

Effect of feed intake

Following delivery to the rumen, drug residence time — as it is absorbed from the rumen and as it flows to more distal sites for absorption by host and parasite — largely depends on the flow rate of the digesta. The inverse relationship between feed intake and digesta residence time (Kay, 1986) ensures that high food intake, particularly with high water content, increases gastric transit rate and reduces drug duration in the gastrointestinal tract (GIT). Conversely, reducing feed intake slows digesta flow and prolongs absorption and recycling of drug between the bloodstream and GIT, in turn, increasing the duration of "toxic" concentrations.

Reducing or withdrawing feed from sheep and cattle for 24h before treatment increased the availability and efficacy of OFZ (Ali & Hennessy

1995), ABZ (Lanusse, Sanchez & Alvarez 1994; Hennessy, Ali & Sillince 1995) and IVM (Taylor, Mallon, Blanchflower, Kennedy & Green 1992; Ali & Hennessy 1996). Field trials showed that efficacy of fenbendazole (FBZ), even against BZ resistant strains of gastrointestinal nematodes of sheep, can be increased by reducing the animal's feed intake for 24 hours before drenching (M. Barret & F. Jackson personal communication).

Removal of feed from sheep before treatment can also significantly affect the behaviour of the salicylanilide, closantel (CLS). This anthelmintic is widely used in strategic parasite treatment programmes, where its sustained availability, bound to plasma albumin in the bloodstream, maintains activity against *H. contortus* for some 4 weeks at a dosage rate of 7.5 mg/kg. However, orally administered CLS is poorly absorbed and about half of the dose is excreted in the faeces within 36 hours of administration (Michiels, Meuldermans & Heykants 1987). Slowing digesta flow rate by withdrawing feed for 24 hours before treatment increases the duration for absorption, resulting in a higher maximum CLS concentration in the bloodstream for a longer period of time. Consequently, the period of protection against the establishment of *H. contortus* larvae is extended to 6 weeks after drug administration, compared with only 3–4 weeks if sheep are consuming a full diet when treated with the anthelmintic (Hennessy & Ali 1997). When used at strategically critical times of the year the extended protective period following CLS administration after feed reduction can significantly reduce the potential for clinical haemonchosis.

Feed restriction is clearly a very useful tool for improved efficacy against resistant strains, but it is arguably of even greater value in situations where the anthelmintic is still "fully" efficacious. By maximising drug action at this early stage of the product's therapeutic "life", the inevitable development of resistance may be slowed.

Dosage regimen

In attempting to increase drug activity the initial response is often to elevate the dose rate. In addition to probably contravening registration conditions, it will also be of little value for BZ or ML compounds which rely on duration of presence, rather than high concentration, for activity. Doubling the dose rate of OFZ results in

a higher maximum concentration and greater concentration-with-time-curve of OFZ in the bloodstream (Sangster *et al.* 1991). However, these and other related studies have shown that the period of maximum concentration and of "toxic" concentrations (that is, concentrations midway between zero and maximum) are not significantly different between single and double dose rates. This most likely explains why Sangster *et al.* (1991) observed similar efficacy of OFZ when it was administered at 5, 10 and 20 mg/kg to goats, which were naturally infected with BZ-resistant worms.

However, multiple dosing at the recommended dose rate at intervals based on the drug's approximate half life can significantly enhance efficacy. Sangster *et al.* (1991) demonstrated that a bolus administration of up to 8 times the recommended dose rate of OFZ did not significantly increase efficacy against BZ-resistant nematodes in goats over that obtained with the single therapeutic dose. But by administering twice the recommended dose of OFZ, divided into three doses of 5, 2.5 and 2.5 mg/kg, each separated by 12 hours (the approximate half life of OFZ) 100 % efficacy against the BZ resistant worms was achieved. Similarly, when goats infected with BZ-resistant nematodes were drenched with three recommended doses of ABZ, each separated by 12 h, Rolfe, Evers & Searson (1994), obtained 100 % reduction in faecal egg count, compared with only 4 % after a single dose of ABZ.

Sustained administration

Rather than using multiple large doses, anthelmintic efficacy can also be increased, even against resistant worms, by sustained low level administration. Kwan, Gyurik, Freeman, Chimes, Ritch & Theodorides (1988) infused the equivalent of a single oral dose of ABZ intraruminally over a 5 day period and increased the efficacy against BZ-resistant *H. contortus* in sheep by over 4 times, compared to a single bolus. It has also been shown that the establishment of infective larvae of BZ-resistant nematode populations of sheep (Anderson, Barton, Hennessy, Page & Steel 1988; Boisvenue, Colestock & Hendrix 1988) can be significantly reduced by prolonged intraruminal delivery of very low doses of a BZ drug.

In laboratory and field trials Knox (1995) demonstrated successful control of BZ-resistant

strains of worms in sheep and goats at average intake rates of 0.5 and 0.75mg FBZ/kg/day respectively, when FBZ was formulated into urea-molasses feed supplement blocks. Daily consumption of the block maintained circulating FBZ metabolite concentrations (Knox, Steel, Ali & Le Jambre 1995) and this mode of delivery is unquestioningly efficient, non labour intensive and inexpensive. Not surprisingly, block delivery of anthelmintic is being applied successfully in developing countries, where performance is compromised by continuous exposure to parasites, while urea supplementation of low quality fibrous feed is used to enhance productivity (Knox & Steel, 1996).

A more expensive sustained treatment option utilises the intraruminal controlled release capsule (CRC). Without increasing the rate of development of resistance (Barnes & Dobson 1990), when integrated with grazing management programmes, the controlled low level release of anthelmintic significantly reduces parasite burdens and increases animal productivity (Barger, Steel & Rodden 1992), compared to single, strategic dosing regimens. The best known products are the Captec Proftril and Extender CRC devices which release ABZ at a rate of 0.5 mg/kg/d over 100 days. Orally administered to sheep using a specially designed applicator, it is well tolerated and highly efficacious against a susceptible nematode population, although the real value of the product lies in the ability to inhibit establishment of incoming BZ-susceptible and resistant larvae.

The same mechanical Captec CRC device has been recently charged with IVM and is now commercially available as the IVOMEC Maximiser™, delivering IVM at a dose rate of 20 µg/kg/day to sheep weighing 20–80kg. The device is likewise well tolerated and, while being spectacularly efficacious against existing infections of all adult and fourth larval (L4) stages of sheep parasitic nematodes, it also provides excellent prophylactic efficacy against ingested nematode larvae and has additional activity against *Psorergates ovis*, *Oestrus ovis*, *Melophagus ovinus*, *Psoroptes ovis* and *Lucilia cuprina* fly strike (Ivomec Maximizer, Technical Manual, 1997).

Directing the dose

Little thought has been given to mechanism(s)

that can direct the dose efficiently to the parasite. Some type of "targeting" formulation will be needed to supply sufficient quantities of drug (metabolites) to the parasite for long enough to give the drug the best chance to be efficacious. One method of achieving this, is to exploit salts of different solubility. For example, pyrantel citrate that must be present in the gut lumen to be efficacious against GIT parasites of pigs, shows low anthelmintic activity in these animals, because it has such high aqueous solubility. Particularly in the case of *Oesophagostomum dentatum* and *O. quadrispinulatum* infection, little of the orally administered citrate salt reaches their predilection site in the large bowel (Bjorn, Hennessy & Friis 1996), and the consequent sublethal exposure is believed to have contributed to the development of pyrantel resistance in these species. Furthermore, the extensive absorption of this citrate salt may be toxic to sows. By using the more insoluble pamoate salt, Bjorn *et al.* (1996) reduced pyrantel absorption and the greater availability in the large intestine significantly increased efficacy against parasites at this site.

On the other hand, even though large proportions of orally dosed BZ drugs do reach the distal GIT, they are poorly absorbed by both host and parasite, as they have very low aqueous solubility and associate strongly with digesta particulate matter (Hennessy *et al.* 1994). However, formulating the OFZ as an amorphous crystalline structure increases its aqueous solubility and absorption, and thus its efficacy, compared with the "conventional" form (Chick, Ali & Emmanuel 1988). The amorphous OFZ has limited aqueous stability and quickly returns to the "conventional" crystalline form, but by delivering it in an oil-based vehicle largely overcomes this problem since, as with its conventional crystalline form, there is greater systemic availability when administered in oil (Ali & Chick, 1992; Hennessy, unpublished results).

A slightly more advanced carrier system comprises incorporating LEV into hydrated aluminosilicate (zeolite). While orally administered LEV is rapidly absorbed in sheep, reaching maximum concentrations in the bloodstream within 3 h (Bogan, Marriner & Galbraith 1982), Shaker, Dyer & Storey (1992) prolonged the therapeutic concentrations and significantly increased the efficacy of LEV against *Nippostrongylus brasiliensis* in rats by

administering the drug in a LEV-zeolite matrix. This formulation, which may also be applicable to livestock, provided a short-term, slow-release formulation that made the drug available for longer, throughout the rat GIT.

Parenteral administration

The field of parenteral administration — both injectable and topical (pour-on) delivery — has recorded the most notable advances in formulation. Much of this progress has been motivated by a desire for convenience of use, coupled with minimal risk of injury to user or animal. It also removes the uncertainties of subtherapeutic action related to poor oral drenching technique, oesophageal groove closure and digesta flow effects. In the case of topical delivery the presence of wool and wool grease provides a considerable chemical and physical barrier that largely precludes this route of administration from sheep, and restricts its use to cattle.

Formulations that improve pharmacokinetic behaviour of pour-on preparations depend on interaction of active ingredient and vehicle with the skin, making the physicochemical properties of the active, and particularly the vehicle, crucial to the success of percutaneous delivery and distribution of active into subcutaneous depots. In turn, drug release from these depots is inversely proportional to the affinity of the active for the fat/tissue into which the drug is dispersed and the rate of diffusion of drug through the depot (Shah, Behi, Flynn, Higuchi & Schaefer 1992).

An initial pour-on LEV formulation for cattle (Dorn & Federmann 1976) was only moderately successful, as high dose rates were required, and the response was variable, being influenced largely by temperature (Forsyth, Gibbon & Pryor 1983). Sufficient percutaneous absorption was obtained of substituted BZ compounds incorporated into tapes impregnated with polyethylene glycol/polyvinyl pyrrolidone and applied to the skin of hamsters, to be efficacious against GIT parasites (Gupta, Srivastava, Katiyar, Jain, Singh & Sarin 1992), but the need for intimate skin contact and the dosages required significantly limit their use in livestock.

Despite its hydrophobic nature previously considered to preclude topical delivery, recent innovations have enabled even OFZ to be

formulated as a pour-on by finely micronising and electrostatically charging the OFZ particles, and coating them with lipid. The manufacturers claim the microemulsion facilitates dermal transportation and yields persistent OFZ availability. They suggest that the skin acts as a reservoir, releasing OFZ into the bloodstream over a period of 4–5 days. The formulation is highly efficacious against most cattle nematodes, including BZ-resistant strains of *Ostertagia ostertagi* and *Cooperia* spp. (Bomatak Technical Dossier, Bomac Industries 1997).

The ideal candidates for parenteral delivery are the highly active and lipophilic ML compounds, which are highly efficacious at very low doses. IVM is injected in a non-aqueous propylene glycol/glycerol formal vehicle, which produces a sustained presentation with a biological half-life of about 8.5 d in sheep (Lo, Fink, Williams & Blodinger 1985), compared to only 2.5 d when sheep are drenched orally (Marriner, McKinnon & Bogan 1987). Topically applied IVM formulation uses a similar lipid/alcohol vehicle to facilitate transdermal passage and extended residence.

The related ML, doramectin (DOR) uses a lipophilic carrier for parenteral administration. By increasing the oil content of the vehicle with sesame oil/ethyl oleate Wicks, Kaye, Weatherley, Lewis, Davison, Gibson & Smith (1993), produced a 50 % increase in the bioavailability of DOR administered by subcutaneous injection in miglyol, with high enough plasma concentrations to be vermicidal, being sustained for some 12 days.

On the other hand, the absence of the disaccharide substituents in the milbemycin, moxidectin (MOX), apparently contributes to significantly greater lipophilicity of this compound, as MOX is more than 100 times more lipophilic than IVM (Hayes 1995). This intrinsic characteristic facilitates efficient transdermal passage, with the result that, without the need of a complex oil-based "carrier" formulation, MOX is extensively deposited in subcutaneous fat reserves, from which it is released so slowly that it protects against nematode infection for up to 35 days, compared to less than 14 days for an equivalent IVM dose.

CONCLUSION

The escalating cost of developing and register-

ing new classes of broad spectrum anthelmintics for use in food producing animals makes it unlikely that any such compounds with unique modes of action will be available in the foreseeable future. Even though in many countries the control of internal parasites of particularly sheep and goats is becoming difficult because of increasing incidence of drug resistance, it has not passed the point of no return. While we cannot continue to indiscriminately use anthelmintic compounds which will result in further development of resistance, we can exploit existing actives by understanding and integrating host physiology and anthelmintic disposition to optimise the activity of existing preparations and minimise actions that compromise drug efficacy.

When using anthelmintics in the field, optimal use requires:

- ◆ knowing the resistance status of the parasite before the most effective drench or drench combination can be prescribed
- ◆ implementing regional strategic control programmes, integrated with the most appropriate anthelmintics
- ◆ administering the drench correctly to ensure maximum entry to the rumen
- ◆ withdrawing feed for 24 h to slow digesta flow rate and to prolong drug availability, before orally administering BZ and ML compounds
- ◆ selecting the most appropriate form of the active ingredient to ensure maximum availability of drug at the site of parasite predilection
- ◆ adopting a dosage or delivery regimen which maximises the effectiveness of the compound against the target parasites.

The "take home" message is that we must use our valuable anthelmintic compounds intelligently if we wish to slow the development of widespread resistance. Adopting these relatively straightforward and practical strategies and procedures will immediately enhance and help to sustain helminth control.

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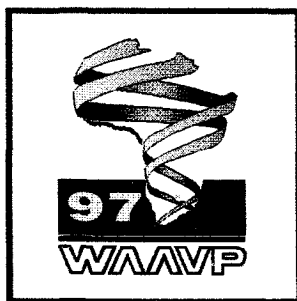
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ABSTRACTS
OF PAPERS AND POSTERS

Development and validation of a geographic information system for *Schistosoma* risk in Kafr El Sheikh governorate, Egypt

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Field studies at 15 sites in Kafr El Sheikh governorate were done to test the validity of AVHRR satellite-derived diurnal temperature difference (dT) maps as an indicator of the suitability of the environment for local snail hosts and *Schistosoma* development-transmission at the local village level. Sites were selected that were representative of thermal-hydrology domains previously defined by dT as very wet, moist, dry and very dry zones. Snail host distribution and *Schistosoma* infection rates were mapped (2km diameter) by spring, summer and fall survey of all canals and drains (100m intervals) at each village. Data was collected on soils, depth to water table, salinity and surface water characters that may affect suitability for snail hosts or *Schistosoma* propagation. Analysis suggests high risk sites have high water table and medium-low salinity. Wet zones had more abundant infected *Biomphalaria* populations/m than drier zones with high dT. Water quality parameters on pH, salinity and dissolved oxygen were not correlated with infected snails/m. ERDAS Imagine and ArcView 3 software were used to construct a GIS from AVHRR dT maps, a May, 1990 Landsat TM scene, point location data on 110 rural health units and point attribute data consisting of human infection rates in 1991-95 Ministry of Health control program records. There was less relationship of dT to human prevalence than was found for infected snails/meter, probably due to success of ongoing treatment, snail control and education programs as well as to variable water contact-water contamination factors.

The pharmacokinetics of moxidectin in sheep

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The close relation between the pharmacokinetic behaviour and the resultant clinical efficacy of antiparasitic drug has been well documented in the last few years. Moxidectin pharmacokinetics and metabolism have been reported in cattle; however, data regarding its pharmacokinetics in sheep remain elusive. The present study was designed to determine the plasma profile of moxidectin given as a 1 per cent injectable solution or a 0.1 per cent oral drench (both at a dose rate of 0.2mg/kg).

Ten laeune sheep were allocated in two groups receiving either injectable or oral solution of moxidectin. The drug was assayed by means of a HPLC method using fluorescence detection.

Moxidectin was detected in plasma between 1h and 60 days post treatment. After oral administration, the C_{max} (28.07ng/ml) and T_{max} (0.22 days) reflected a faster way of absorption compared to subcutaneous administration (8.29ng/ml and 0.88 days respectively). There were no difference in the area under the concentration time curve (AUC). The mean residence time (MRT) resulted higher for subcutaneous route (16.8 days) compared to that of oral route (12.55 days). This difference results in a substantial broadening of the plasma concentration and persistence of the drug in the case of subcutaneous administration, for example subcutaneous route allows plasma concentration higher than 2ng/ml during 18 days and oral route only during 8 days.

This experiment showed the influence of administration route on the pharmacokinetic parameters of moxidectin. The results are well correlated with the corresponding efficacy of the two formulations.

Evaluation of the efficacy of an ivermectin controlled-release capsule against *Psorergates ovis* in sheep

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A single oral or subcutaneous dose of ivermectin (IVOMEC® Liquid for Sheep or IVOMEC Injectable for Sheep, respectively) given at the recommended dose rate of 200mcg/kg is very effective for the control of *Psorergates ovis* in sheep. Ivermectin has now been formulated in an intra-ruminal sustained release device for sheep from which drug is delivered at a minimum dose of 20mcg/kg/day for 100 days. Two trials were conducted to confirm the efficacy of ivermectin delivered from a controlled-release capsule (CRC) against natural infestations of *P. ovis* in sheep. In each trial 16 adult Merino sheep were used. In the first trial the sheep weighed 35.7 to 54.0kg on Day -1 and were ranked by pre-treatment mite counts and fleece derangement scores and allocated consecutively to replicates of two animals. In the second trial the animals weighed 41.5 to 57.2kg on Day 0 and were ranked by pre-treatment mite counts within two fleece lengths and allocated consecutively to replicates of two animals. Animals within each replicate were randomly allocated to either an untreated control group or a group receiving a single CRC delivering 1.6mg of ivermectin per day. In both studies, personnel recording data were blinded to the assignment of treatment groups. Skin scrapings were performed for mite counts before commencement of the trials and on Days 7, 14, 28 or 29, 56 and 84. Sheep were housed indoors in the first trial with sheep in separate pens for each group. Individual outdoor pens were used in the second trial. In the first trial no live mites were recovered from CRC-treated animals from Day 14 onwards. Four of the control animals remained infested with mites with counts ranging from 1 to 58, on Day 84. In the second trial no live mites were recovered from any CRC-treated animals from Day 56 onwards. Five of the control animals remained infested with mites, with counts ranging from 6 to 149, on Day 84. The results of these studies demonstrate that ivermectin delivered by intra-ruminal CRC at a minimum dose of 20mcg/kg/day is efficacious against *P. ovis* in sheep.

Use of GIS in planning a helminth control programme in KwaZulu-Natal

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A human helminth (intestinal nematodes and schistosomiasis) control programme is being planned for the province of KwaZulu-Natal. However, while much is known about the distribution of schistosomiasis in the province, very little is known of the distribution patterns of the common intestinal nematodes. To redress this a method combining the use of simple ecological transect surveys and GIS was developed to produce maps of KwaZulu-Natal showing (i) the likely distribution of these parasites and (ii) zones of differing priority for control. When coupled with data on the distribution of primary schools in each zone and the number of pupils per school, the number of children to be treated in each zone could be estimated. This in turn allowed the development of different control strategies for the different zones within the province and estimations of the costs of each.

Repellency and antifeeding effects of three antiflea products : pilot test F. ASCHER¹, P. BOURDEAU², J.P. BOYD³, V. GOFFAUX⁴

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Since allergic dogs react to flea bites of even a few fleas, the objective of this pilot test was to check the antifeeding effect combined with the immediate and residual repellency effects of 3 antiflea products on fleas that have been given the opportunity to infest the animals. After having been checked for flea attractiveness, 8 Pointer dogs (mean weight : 19.3 kg) were split into 4 groups of 2 each : one non-treated [CTL] and 3 groups treated once either with a 0.02% pyriproxyfen + 2% permethrin spray [DUO] (Duowin[®], Virbac[®], 5 ml/kg), a 0.25% fipronil spray [FRL] (Frontline[®], Rhône Mérieux[®], 3 ml/kg), a 9.1% imidacloprid solution [ADV] (Advantage[®], Bayer[®], 2.5 ml/dog). The following test design was used for each dog to simulate a naturally occurring flea population exposed to and infesting a host animal. Weekly for 6 weeks, 100 adult fleas were released into a repellency box and allowed to acclimate before a caged dog was placed into the box for a 10 min. exposure period. The dog was then transferred for 30 min to an adjacent second box free of fleas. Fleas found in the two boxes and on the dog 30 min. post exposure were counted and bioassayed to determine if they had ingested blood. Neither CTL, FRL nor ADV provided an effect on fleas. However, during this 40 minutes period, a high number of fleas left the animals of the DUO group throughout the 6 week period, thus preventing a high number of fleas from feeding. The early and strong repellent and antifeeding effects of DUO over an extended period support its use for control of FAD.

group	Repellency effect : % of fleas found on the dogs 40 min. after flea exposure started					
	1	2	3	4	5	6
CTL	93	89	96	98	96	93
DUO	2	7	8	15	30	55
FRL	85	92	99	98	96	97
ADV	82	89	91	76	95	92

group	Antifeeding : effect of early repellency on blood consumption									
	box 1		box 2		body		total		fed fleas (%)	
	W1	W2	W1	W2	W1	W2	W1	W2	W1	W2
CTL	5	4	1	2	10	6	16	12	84	88
DUO	62	51	14	23	2	3	77	78	23	22
FRL	7	4	3	1	12	12	22	17	78	83
ADV	7	7	7	2	14	8	27	17	73	83

W1, W2 : 1st and 2nd week post treatment

Knock-down and antifeeding effects of antiflea products : pilot test

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Since allergic dogs can react to flea bites, the objective of this pilot test was to check the knock-down [KD] and antifeeding [AF] effects of 3 antiflea products. 8 Pointer dogs (mean weight : 19.3 kg) were split into 4 groups of 2 each : one non-treated [CTL] and 3 groups treated once either with a 0.02% pyriproxyfen + 2% permethrin spray [DUO] (Duowin[®], Virbac[®], 5 ml/kg), a 0.25% fipronil spray [FRL] (Frontline[®], Rhône Mérieux[®], 3 ml/kg), a 9.1% imidacloprid solution [ADV] (Advantage[®], Bayer[®], 2.5 ml/dog). Each dog was infested with 100 adult unfed *Ctenocephalides felis* each week for 6 weeks. Fleas that fell off the dogs after their release along the backline were counted at 15, 30, 60, 120 and 240 min. intervals. 4 hours after each infection, the fleas present on the dogs were counted and removed. All fleas were bioassayed to determine if they had ingested blood. DUO provided an early and strong knock-down during the first two weeks with more than 90% of the fleas fallen off within 15 minutes. Afterwards this effect began to decrease but DUO was the only product through week 6 to provide more than 90% knock-down in less than 4 hours. The early and strong knock-down and antifeeding effects of DUO support its use for control of FAD.

group	week post-treatment					
	1	2	3	4	5	6
knock-down : % of fleas that fell off the dogs within 1 hour						
CTL	6	4	3	1	5	0
DUO	94	99	96	91	78	83
FRL	7	15	6	4	1	3
ADV	8	8	11	2	6	4
antifeeding : % of fleas that fed within 4 hours						
CTL	95	96	98	96	96	98
DUO	4	3	13	20	45	36
FRL	89	73	46	75	82	84
ADV	92	85	87	83	87	91

Computer-assisted reconstruction of the amphidial neurons of ivermectin-susceptible and ivermectin-resistant strains of *Haemonchus contortus*

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Ivermectin-resistant strains of the nematode *Caenorhabditis elegans* have abnormalities in the structure of their amphids, or amphidial neurons. On the hypothesis that IVM-resistance in the sheep parasitic nematode *H. contortus* might also involve abnormalities in amphidial ultrastructure, the amphids of 3 pairs of worms from (1) a field strain from Louisiana, (2) the Merck IVM-susceptible parental strain, and (3) the Merck IVM-resistant daughter strain were reconstructed from serial electron micrographs. Thirteen dendritic processes are found in the amphidial channel in all three strains, however, since two of the amphidial neurons end in double ciliated dendritic processes, 11 neurons receive signals from the external environment. Two additional processes leave the amphidial channel, and end in the amphidial sheath cell. These appear to be homologues of the "finger cell" and one of the "wing cells" of *C. elegans*. In the field strain and the Merck IVM-susceptible strain all of the dendritic processes in the amphidial channel end immediately posterior to the amphidial pore. In the IVM-resistant strain, however, only two processes extend to the pore; all of the remaining nine are much shorter, ending deep within the amphidial channel.

Preliminary study of the parasitism pattern of goats in the family sector in Mozambique

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A survey in order to study the geographical and seasonal patterns of the occurrence of intestinal parasites and haemoprotozoa of goats is being carried out in four of the ten provinces of Mozambique. This paper deals with the results obtained during November 1996 to March 1997, the first five months of the 12-month survey.

A total of 1 506 faecal samples were analysed. The modified MacMaster technique was used for nematodes and *Eimeria* spp. on all these samples, and 1389 were subjected to a sedimentation technique to determine the number of trematode eggs. In addition 1 624 blood smears, stained with Giemsa's stain, were examined for haemoprotozoa.

The prevalence of trichostrongylid eggs was 32.4% during November 1996, 28.8% during December 1996, 74% during January 1997, 77.5% during February 1997 and 59.9% during March 1997. The means of the egg counts decreased during November and December 1996, but increased in January 1997 to 2 384 eggs per gram faeces (epg) in Maputo, and in February 1997 to 978 in Gaza, 3 393 in Tete and 936 in Cabo Delgado. The prevalence of *Eimeria* oocysts increased from November (56.9%) to reach a peak during February 1997 (93.2%), but decreased during March 1997 (69%). Only 377 (27%) of the specimens examined were positive for trematode eggs. Haemoprotozoa were present in 50.2% of blood smears. Of these, 416 (51%) had *Theileria* spp., 373 (45.7%) *Anaplasma ovis* and 27 (3.3%) a mixed infection of these protozoa. The prevalence of the haemoprotozoal infections did not differ much between the provinces, it being 48.8% in Maputo, 53.9% in Gaza, 43% in Tete and 50.6% in Cabo Delgado.

Although the survey is still in its early stages, the preliminary results indicate that the parasites mentioned above play a significant role in the health and productivity of goats in the family sector.

Monoclonal antibody against *Babesia equi*

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Equine babesioses are caused by *Babesia equi* and *B. caballi* and are widespread throughout world. Although a large number of serological tests have been used for the detection of anti-babesia antibodies, none has been specific for accurate diagnosis. Therefore, development of diagnostic tests with high sensitivity and specificity is needed. In the present study, monoclonal antibodies (MAB) were made against *B. equi* for purification of diagnostic antigens. Culture-adapted *B. equi* was used for immunization of BALB/c mice. Spleen cells from immune mice were fused with Sp2 myeloma cells. Hybridoma culture supernatant was screened for antibabesial activity by indirect fluorescent antibody test (IFAT). Class and subclass of MAB were determined with a mouse monoclonal isotyping kit. Nine clones were obtained after second screening and one clone (1.11.B2.G3) was selected for further analysis after third cloning. This MAB is *B. equi* specific and did not react with *B. caballi*, *B. ovata* and *B. microti*. Subclass of the MAB 1.11.B2.G3 is IgG1 and this MAB reacted with parasite membrane and recognized 19kDa protein of *B. equi*. Addition of MAB 1.11.B2.G3 to culture fresh medium (500 µg/ml) inhibited parasite multiplication in vitro.

011

Conducting epidemiology studies on helminthiasis amongst smallholder farms - problems and possible solutions from Kenya

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Difficulties exist in evaluating the epidemiology of helminth infections in the ruminant livestock owned by poor smallholder farms in developing countries. Unlike in most studies conducted in the West, statistically significant sample sizes cannot be found within individual farms where average flock/herd sizes are very small. Increasing the number of farms adds the problems of drawing up suitable sampling frames, conducting suitable farm stratification, assessing differences in management between units and ensuring that all the chosen farms lie within a homogeneous climatic boundary. The demands of statistical accuracy very soon meet up against the limits of logistics and funding.

Very little literature exists on how best to approach studies in these areas, yet increasingly, development projects must attempt the task. Often (as in our case) the scientists in these projects lack the skills in biometrics and classical epidemiology that they would need to ensure that the results of their studies are meaningful.

Attempts in Kenya to study helminth epidemiology in different parts of the country have found that, depending on flock size, up to 140 farms require to be sampled on a monthly basis in order to make realistic comparisons between treatments.

010

Fish parasites - a first level screening system for heavy metal pollution?

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Water-pollution has become an international problem. Chemical water quality testing is becoming increasingly expensive and is accurate only at the instance of sampling, therefore alternative methods for determination of water quality is currently investigated. Biological indicators are favoured as it reflects long term exposure as well as acute toxicity. Fish, being near the top of the food chain is regarded as representative of fluctuations in aquatic health.

A model for fish health was tested in the Olifant River. This river has a history of pollution. A control site was selected in the vicinity. Water quality, health index indicators and metal accumulation was determined and data regarding the occurrence and distribution of parasites was sampled.

Results of these surveys indicated that the general health of the fishes was, as expected, negatively influenced by the weakened environment. It was furthermore shown that the parasite composition differed markedly. In the less polluted environment ectoparasites were abundant and endoparasites almost absent. In the more polluted environment, however, endoparasites flourished and ectoparasites were present in low numbers.

The feasibility of using this phenomenon as an early warning system, which can be used by water resource managers is currently evaluated.

012

Genetic resistance to helminths in cattle, sheep and goats

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Between- and within-breed genetic variation for resistance to gastrointestinal (GI) nematode parasites in sheep and cattle is well documented and in Australia and New Zealand commercial sheep breeding schemes are operational which include resistance to endoparasites along with production traits. Evidence for genetic resistance to GI nematodes in goats is fragmentary and inconsistent and further research is warranted. There have been a few reports of breeds of sheep which are resistant to liver flukes (both *Fasciola gigantica* and *Fasciola hepatica*).

Important issues which still have to be resolved include: identification of alternative predictors of resistance in addition to the commonly used faecal egg counts and packed red cell volume - this might include immunological parameters or molecular genetic markers; more precise estimates of the genetic correlations between production traits, resistance to GI nematodes and resistance to other economically important diseases; calculating relative economic values for resistance to endoparasites; assessment of gains in profitability that might be expected from breeding for resistance; and development and testing of integrated helminth control strategies which might include resistant hosts, strategic drenching, grazing management, strategic nutritional interventions, biological control, and in the longer term, vaccines.

The eggs of the warthog louse (*Haematopinus phacochoeri*) : Scanning electron microscopic observations

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The large eggs of *Haematopinus phacochoeri* are found attached to the long dorsal mane bristles of the diurnal warthog, and are generally exposed to great environmental stresses such as excessive sunlight, temperature and the occasional plunge under water during grooming. This study was therefore undertaken to determine how these eggs are adapted to tolerate the various environmental changes it is constantly exposed to.

Specimens attached to the bristles were fixed in 70% ethanol, whereafter they were routinely processed for scanning electron microscopic observation in a Leica Stereoscan 420 SEM.

The shell consists of two layers, i.e. the endo- and exochorion. These two layers are attached at the pre-formed line of weakness at the operculum and again near the aeropyle. Elsewhere these two layers are completely separated by the respiratory layer.

The aeropyles extend through all of the thick exochorion where they assist in the movement of gas to and from the respiratory layer. The aeropyles terminate as round openings on the inner side of the exochorion, from which radiate a system of ridges arranged in polygons of 5-6.

The operculum consists of a meshwork of air-containing chambers. Each micropyle opens into an air-filled chamber surrounded by distinct vertical columns that function to provide support to the structure. A spiked, disc-shaped embryonic egg burster was observed on the cranial side of the embryonic cuticle under the operculum.

In order to emerge from the shell, the larva increases the internal pressure until the operculum is forced open along the pre-formed line of weakness. The extensive meshwork of air-chambers found in the exochorion may protect the larva against extremes of sunlight and temperature, while the aeropyles ensure a sufficient supply of air and water.

When all the abovementioned are considered, it is quite evident that the eggs are micro-structurally adapted to enable the larva to survive the harsh diurnal environment on the bristles of the warthog.

Study of the horse parasites cyathostomes (Strongylidae, Cyathostominae) in the São Paulo State, Brazil.

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By previous fecal examinations 14 naturally nematode infected equine hosts were selected from horse-breeding farms in Jaboicabal County, São Paulo State, Brazil. Such animals were classified into two experimental groups according to their ages: seven animals, 12 to 24 months old, were allotted to the young group and other seven equines, all of them aging more than 60 months, constituted the adult group. One week later all the fourteen equine hosts were slaughtered and the separate contents, plus a through scraping of each section of their gastro-intestinal tubes were collected. Nematodes from every segment were identified, sexed and counted, and special attention put on the Cyathostominae of the large intestine, whose main species were carefully studied by means of an electronic scanning microscope. From the total of 537128 collected nematodes from the fourteen animals of both age groups 80,5% belonged to Cyathostominae (small strongyles). From these, 72,64% came from the young group hosts and the remaining 27,36% from the adult one. Within each host age group, the "small strongyles" added up to 97,3% in the young group, while in the adult group they kept at the 74,1% level. The hosts of the young group harbored 22 species of "small strongyles", while those of the adult group kept only 14 species. *Cylicostephanus longibursatus*, *C. goldi*, *Cyathostomum catinatum*, and *Cylicocyclus insigne* were frequently identified. The electronic scanning microscope showed itself a rather useful technical instrument in the study of morphological aspects of the Cyathostominae species. As a general synthesis this study points out the higher frequency of the "small strongyles" among the nematode parasites of equines at the São Paulo State, where it prevails above all other nematode species, both in young and adult hosts.

HAEMONCHUS PLACEI INTESTINAL PROTEINS AS HOST PROTECTIVE ANTIGENS. L.R. Ballweber, C. Siefker. College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762, USA.

The protective value of *Haemonchus placei* intestinal proteins was examined in Holstein bull calves. Five calves received 100 µg intestinal protein in 5% dextran-sulfate/PBS by subcutaneous injection on Day 0 (14 weeks of age). At the same time, five control calves received a similar injection of PBS. Injections were repeated on Day 27 and, on Day 55, all calves received approximately 3300 infective *H. placei* larvae. Serum antibody levels and counts of nematode eggs per gram of feces were determined throughout the study. Calves were necropsied at 7 weeks post-challenge for nematode recovery.

Significant increases in log (10) titers of antigen-specific serum IgG1 and IgG2 were detected in the treated animals ($p \leq 0.05$). Nematode egg shedding was intermittent and, on days 26-49 post-challenge, significantly lower ($p \leq 0.05$) than in controls. There was no significant difference between groups in total number of nematodes recovered. However, significantly more ($p \leq 0.05$) developing fourth-stage larvae and significantly fewer ($p < 0.001$) adult females were present in the treated calves than in controls. Lengths of adult males and females were significantly shorter ($p < 0.001$) in treated calves. Protection, as evidenced by decreased egg shedding and fewer females, was correlated with increased mean post-challenge IgG1 and IgG2 titers. Vaccination-induced decreases in nematode egg shedding could result in decreased pasture contamination. Thus, vaccination protocols may become an adjunct to strategic parasite control programs for the bovine.

Pathophysiological alterations of Holstein-Friesian calves prime-infested with the tick *Boophilus microplus*

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In order to study metabolic alterations of Holstein-Friesian calves infested with the Mozo strain (free of hemoparasites) of the tick *Boophilus microplus*, nine calves aged 6 months were lodged in individual stalls since 3 days of age, where they were maintained free of endo and ectoparasites until the beginning of the experimental period. The experiment was designed to distinguish the effects caused by reduced food intake and by the ticks specific effect. One group of 5 animals was experimentally infested with approximately 20.000 tick larvae (1g of tick larvae) and with a controlled feed offer. A second group of 2 animals was kept tick-free and pair-fed to 2 animals of the tick-infested group. A third control group of 2 animals was kept tick free and had the same feed offer of the infested group. The peak of engorged-females fall was at day after infestation (DAI) 23 with an average of 1113.2 ± 160.8 and the mean total number was 4689.8 ± 476.4 . Before, during and after the infestation, several metabolic parameters were evaluated, demonstrating that: the specific effect was responsible for a significant lower weight gain, increased body temperature from DAI 17 to 28, decreased significantly the haematocrit, erythrocyte and haemoglobin levels since DAI 21, not reaching the control group levels until DAI 42. Lymphocytes and leukocytes were increased significantly in DAI 28. In infested animals was also detected a tendency of drinking more water and eat less food in DAI 19 to 22 and from DAI 17 to 20 respectively. The pair-fed group animals did not significantly differed of control group animals in all parameters. The nitrogen balance of infested animals, bone marrow response, and serum alterations are under analysis. As this strain is free of hemoparasites and the anorectic effect, observed between pair-fed and control groups, did not altered significantly the hemogram, the tick *B. microplus* seems to be itself the responsible for blood alterations. The general waste of the hosts showed to be a consequence of the exclusively action of the infestation by *B. microplus* observed in a susceptible Holstein-Friesian calves.

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Zambia and Belgium have co-operated during the past fifteen years to define a suitable control strategy for theileriosis, caused by the protozoon *Theileria parva* and transmitted by the ixodid tick *Rhipicephalus appendiculatus*, in the eastern part of the country. Recently this collaboration was extended to include the Southern and Central Provinces. Immunisation of calves with a single local *T. parva* isolate proved to be the most appropriate control method for the major part of Eastern Province: theileriosis was endemic, causing large losses in the calves only, there was full endemic stability for the other tick-borne diseases and the environment was not favourable for tick populations to become a problem. Immunisation against theileriosis has become a routine operation, carried out by the Veterinary Department, at present on a partial cost-recovery basis. Epidemiological research is currently concentrated on a few epidemic areas, with special interest for vector ecology in this area, which is transitional between the east African polymodal vector phenology and southern African monomodal phenology, beside routine surveys to monitor the further spread of the vector and the disease in the province and follow-ups of sentinel herds to study the influence of the large-scale immunisation on the epidemiology of the disease. The situation in the Southern Province is more complex and immunisation is unlikely to become the sole control method: theileriosis is not endemic in large parts of the area and the danger of spreading the disease through induction of a carrier state after immunisation is real. Recent epidemiological studies may not be entirely representative because of the drought and the introduction of foreign *T. parva* stocks. Precise information on the length of protection offered by immunisation in the absence of natural booster infections is a key parameter in the decision process, but this information is at present wanting.

Distribution of *Ascaris suum* in naturally infected pigs

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The objective of this study was to investigate if a predisposition exists in pigs to infection and reinfection with *Ascaris suum*, by studying parasite distribution and host response in naturally infected pigs.

Fifty helminth-naïve pigs were turned out on a pasture contaminated with *A. suum* eggs. The pigs received anthelmintic treatment after 10 weeks when egg counts indicated patent infections. All pigs were housed individually in cages and their faeces was collected for 3 consecutive days. The expelled worms from each pig were counted, after which the pigs were reintroduced to the infected pasture. After a subsequent 10-week period, all pigs were necropsied and their worm burdens recorded. Blood samples were taken regularly and analyzed by means of an ELISA test. The degree of aggregation of *A. suum* was measured using the negative binomial parameter k .

Heavy overdispersion was observed at treatment ($k=0.16$) and after reinfection ($k=0.77$). The number of pigs that harboured worms increased from 34% at treatment to 84% at time of slaughter. Mean worm burdens did not differ significantly (10.4 versus 9.4), but a significant correlation was found between worm burden at treatment and at necropsy ($r=0.39$, $P<0.01$). Chi-square analysis showed that pigs harbouring worms at treatment were more likely to harbour worms again after reinfection ($\chi^2=4.73$, $P<0.05$).

The results of this study suggest that a predisposition exists in pigs to establishment of adult *A. suum*. Degree and length of exposure and their influence on the immune response, including the ELISA results, will be discussed.

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The regular scientific researches on veterinary helminthology have been carried out in Russia since 1920 initially by Department of Helminthology of the State Institute of Experimental Veterinary Medicine and later (since 1932) in All-Union (All-Russian now) K.I.Skryabin Institute of Helminthology. The investigation of all complex of problems connected with helminths and diseases caused by them as well as developing on base of this knowledge control measures of helminthoses to attain the aim to abolish these infections in animals are among the number of tasks of institute. The directions, instructions, recommendations, methodics, certifications on manufacture of anthelmintics and their drug formulations, diagnosticums, apparatus and instruments are the production of institute. The instruction "Measures on prophylaxis and control of helminthoses of animals" is a fundamental document and it has gone through several editions (1950; 1971; 1981; 1988; 1996 s). In the last revised and supplemented edition (1996) of instruction measures against more than 70 helminthoses of mammals and birds spreaded in Russia are stated. The second basic document "Rules of veterinary inspection of slaughter animals and veterinary-sanitary examination of meat and meat foods" (1983) includes examination on helminthoses especially on helminthozoonoses. The monitoring of situation on helminthoses in country is fulfilled by recording of data on infection and death of animals as a result of diseases according to special forms of veterinary statistic accounts (1-vet; 1-vet A; 4-vet; 5-vet). It's regularity allows successfully reveal new foci of parasitoses and in time to prevent their subsequent spreading.

Parasites nematodes from respiratory tract of ruminants swine and equine in Argentina. Modified perfusion diagnostic technique

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Argentina is one of the countries where infestation by *Dictyocaulus* spp. is widespread. Pneumoparasites are the cause of an important damage in bovines and ovines thus originating a decrease and loss in productivity.

The importance of pneumoparasites was emphasized during IV Argentine Congress of Veterinary Medicine, (1982). The development of investigations on parasitic diseases of the respiratory tract of animals, whose by-products are a source of food, is totally justified in our country. A brief description on parasites species from respiratory system on bovine, ovine, swine and equine in different areas of the country was done.

The diagnostic lung perfusion technique with modification procedures described to obtain rapidity with more simple operation, as well as better fluidity of the perfused liquid.

Climatic impact on gastrointestinal helminthiasis in bovine of 3 Arroyos area (Buenos Aires Province)

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Field studies on the seasonal variations of parasitic gastroenteritis in bovine from Tres Arroyos area were conducted from March 1988 to April 1989. The epizootiological investigations were done in 3 farms with a group of 10 calves each. Female and male aged 7 to 10 month and average body weight between 170-220kgs.

Fecal samples from each animal were collected every month, and processed at the Laboratory to determine the number of nematode eggs per gram of faeces (epg). The intensity of infestation was < 400 epg and > 400 in two and one farm respectively.

Weather information historically showed for the area, that from November to March as a favourable period for survival of free living stages of nematode on the pasture. During our studies this favourable weather conditions were reduced only at December-November and then March.

Atypical climatic conditions for the year, 1988, were observed and clearly demonstrated in relation with the epg seasonal variations.

Weather has an effect on the development, seasonal variations, prevalence and distribution of nematode eggs.

The year 1988 was atypical because of dryness, normal rainfall average per annum is 733.5mm and during our studies was 505.5mm per annum. We continue with the climatic observations until 1996 and the rainfall average from 1988 confirmed the atypical conditions of that year.

[†] in memoriam to Dr De Piero

023

Are scrub hares (*Lepus saxatilis*) the true hosts of some *Trichostrongylus* species of wild ruminants?

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As part of the surveys of the parasites of South African wildlife, scrub hares, *Lepus saxatilis*, were shot each month from August 1988 until August 1990 at Skukuza in the Kruger National Park. A total of 124 animals were examined and their helminths collected, identified and counted.

Impatiens tuberculata (range 10-520, prevalence 32.2%), *Cooperia hungi* (20 in each of two hares, prevalence 1.6%), *Trichostrongylus deflexus* (range 40-9 563, prevalence 96.8%), *Trichostrongylus falculatus* (range 28-2 693, prevalence 48.4%) and *Trichostrongylus thomasi* (range 23-1 796, prevalence 50.8%) were recovered from the hares. These results were compared with those of earlier surveys of kudus, *Tragelaphus strepsiceros* (n=96), examined south and west of Skukuza and impalas, *Aepyceros melampus* (n=142) and warthogs, *Phacochoerus africanus* (n=28), examined at the same locality as the hares. The scrub hares and impalas harboured all five of the above helminth species in common and kudus and warthogs four species. The prevalence of *C. hungi* and *I. tuberculata* was highest in impalas (87.3% and 72.5%, respectively), that of *T. deflexus* and *T. falculatus* highest in scrub hares and that of *T. thomasi* highest in warthogs (75%). This is surprising as *T. thomasi* has always been regarded as a definitive parasite of ruminants. Kudus did not harbour *T. thomasi* and were poor hosts for *C. hungi* and *T. falculatus*, while warthogs did not harbour *C. hungi* and were poor hosts for *I. tuberculata*, *T. deflexus* and *T. falculatus*. In addition, the mean intensities of the infection with these nematodes were invariably higher in the scrub hares than in any of the antelopes or the warthogs.

In Europe, hares and rabbits are successful hosts of, amongst others, *Trichostrongylus retortaeformis*, an important parasite of pheasants. Scrub hares seem to be as successful for the *Trichostrongylus* spp. of wild ruminants in this country. Although no surveys of hares on farms where domesticated ruminants are kept have been undertaken, we assume that scrub hares will be equally good hosts of *Trichostrongylus colubriformis* and *Trichostrongylus axei*. We do not advocate extermination of the hares; however, their role as alternative hosts should be borne in mind when devising control programmes for helminths.

022

Parasites in sea fish capture in the Argentine Atlantic Ocean

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Marine parasites include nematodes, cestodes, trematodes, and protozoa. Argentine use different capture method to get fish from our coasts.

Investigations showed the presence of *Anisakis* sp., *Phocanema* sp. And *Contracaecum* spp. in the fishes *Merluccius merluccius hubbsii*, (merluza); cestodes parasites in *Genyterus blacodes*, (abadejo) and in *Micropogon opercularis*, (corvina). Also, cestodes were found in *Cynoscion striatus*, (pescadilla).

The protozoa *Kudoa rosebuschi* was found in the merluza and the trematode *Dimurus breviductus* in Scombriformes marine fish.

For the future, because of the importance of the fish capture in our country, we must emphasis the increment of research and investigations plan on the ethiological, epizootiological and connected studies.

The sanitary plan, undoubtedly, have to included more budget for this field of animal production.

024

Chemotherapy of fasciolosis today and in the future

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No new effective drugs have been developed for the treatment of fasciolosis for the last decade. For effective chemoprophylaxis, drugs with efficacy against early immature and adult flukes should be used. Due to existing or potential drug resistance, a minimum number of annual treatments with yearly drug rotation should be used, according to strategic programs, based on climatic conditions. More attention should be paid to preventive farm management.

Specific and multiple drug resistance in *Fasciola hepatica* to some anthelmintics, particularly to triclabendazole and salicylanilides, has been demonstrated in the field and in the laboratory. Use of synergistic combinations of drugs from different chemical groups, with some efficacy against immature flukes, is recommended to control resistant flukes and for the potential prevention of the development of resistance in susceptible fluke populations. Some combinations may achieve broad spectrum activity against flukes, tapeworms, some nematode species and other parasites.

Suppression of fasciolosis has been achieved with frequent treatments, resulting in negligible reinfection in some farms, requiring no treatment for up to two years. Is this method a desirable option?

Frequent treatments with a single, effective anthelmintic may result in serious resistance problems, and in a possible reversion to the use of old, less effective and/or toxic drugs.



Efficacy of a closantel/oxfendazole combination against *Fasciola hepatica* in sheep

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Objectives: The efficacy of a new drug combination of closantel (CLOS) and oxfendazole (OXF) against immature *Fasciola hepatica* aged 6 weeks was tested in experimentally infected sheep. **Methods:** the controlled test was carried out in 128 sheep in 4 trials. Sheep were inoculated with 300 metacercariae each, produced in *Lymnaea tementosa*. Fluke strains either susceptible or resistant to salicylanilides were used. All groups were treated 6 weeks, except in 2 groups treated 8 weeks after inoculation. Exp. 1&2, susceptible strain: 76 sheep in groups of 5 or 6 were treated with a combination product at 7.5 and 5 or 10 and 5 mg/kg CLOS and OXF, or with separate formulations simultaneously at the above dose rates. Other groups received CLOS alone at 7.5 or 10 mg/kg and one received OXF alone at 5 mg/kg. 12 sheep in groups of 4 were treated with the combination or CLOS alone 8 weeks after inoculation. Exp. 3&4, resistant strain: 40 sheep were treated in groups of 4 or 6 with CLOS + OXF at 7.5 + 5 mg/kg, CLOS alone at 7.5, OXF alone at 5 mg/kg. In each experiment one group was untreated. All sheep were killed 16 weeks after inoculation, the flukes were recovered and counted. **Results:** Exp. 1&2, susceptible strain: Fluke numbers in all treated groups were significantly lower than those in controls. There was no significant difference between the fluke burdens of the treated sheep. The efficacy ranged 71 to 91%. When CLOS was used at 10 mg/kg the efficacy was slightly higher. The fluke burdens in the OXF alone treated groups were not significantly different from that in controls. In the sheep treated 8 weeks after inoculation the efficacy was higher, 89 to 93%. Exp. 3&4: resistant strains: The reduction in the CLOS and OXF alone group was 34-47% and 0-1% respectively. The reduction in the groups treated with the combination was 71-74%, appreciably higher than the sum of the efficacy of the two drugs used alone. **Conclusions:** The combination products were as effective (around 80%) as the simultaneously given separate formulations of the components against strains susceptible to salicylanilides against flukes aged 6 weeks. More than 90% efficacy was achieved against flukes aged 8 weeks. The combination product was 71 to 74% effective against salicylanilide resistant flukes aged 6 weeks and the results of the experiments suggest a synergistic activity between the two drugs.

A comparison between three sustained release bolus systems to control lungworm (*Dictyocaulus viviparus*) infections in calves and their effect on the build up of immunity

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Five groups of 6 calves were available to test the efficacy of three sustained release bolus systems to control lungworm disease in calves. Group 1 served as control group, group 2 was treated with a Chronomintic[®] bolus, group 3 with a Panacur[®] SR bolus, group 4 with an Ivomec[®] SR bolus and calves of group 5 were vaccinated against lungworm before a Chronomintic[®] bolus was given. All groups were kept on separate pastures of 1 ha. Pastures were not grazed by cattle in the foregoing year. Calves were turned out on May 28 and boluses were given at June 4. Calves received trickle infections with in total 200 lungworm larvae between 7 and 34 days after bolus application. After housing (October 28) calves were challenged with 5000 larvae and slaughtered 3 weeks later. No clinical signs were observed during the study. Weight gain in treated groups was better ($p < 0.05$) than in the control group. Larval output started in the controls between 25 and 32 days after first infection. Peak output was seen after the 2nd generation of lungworms reached maturity (60-80 days after the first dose). In group 2, calves started to excrete larvae at the end of the grazing season. Mean peak output never reached 2 LPG. In group 3, larval output started during housing and was < 1 LPG. No larval output was seen in groups 4 and 5. The results of the ELISA followed exactly the pattern of larval output. Titres above the cut-off value were only seen in groups 1, 2 and 3. After challenge, calves of group 1 were completely immune. Calves with an Ivomec[®] SR bolus had a higher mean total worm count ($p < 0.05$) than calves with a Chronomintic[®] bolus. No differences in worm counts were observed between groups 2, 3 and 5. Worms in groups 5 were retarded in growth ($p < 0.05$), probably an effect of vaccination.

Resistance of immature and adult *Fasciola hepatica* to triclabendazole in the field

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Objectives: High morbidity and mortality due to fasciolosis was reported in a flock of adult merino sheep after repeated treatments with triclabendazole at a farm in an irrigation area of Australia. The cause of the apparent failure of the drug was investigated. **Methods:** Some sheep was examined at necropsy and faecal egg count reduction tests were carried out. Eggs were isolated for incubation and metacercariae were produced in *Lymnaea tementosa*. Exp. 1: 42 sheep were infected with the isolate and a controlled test was carried out using dose rates of 5 to 10 mg/kg 2, 4, 6 and 12 weeks after inoculation in groups of 4. One group each was treated with triclabendazole at 20 and with closantel at 7.5 mg/kg. Exp. 2: 36 sheep were inoculated with metacercariae, originated from a farm where triclabendazole gave good control. Triclabendazole was given at 5 to 10 mg/kg 4, 6 and 12 weeks after inoculation. One group was treated with closantel at 7.5 mg/kg 6 weeks after inoculation. In both experiments the treatments were carried out from the same commercial container of triclabendazole (Fasinex, Ciba-Geigy Australia Ltd.) **Results:** Exp. 1: the number of flukes recovered at necropsy was not significantly different from those of the controls in any of the groups treated with triclabendazole at any dose rates. The reduction in the groups was from 0 to 17%. Treatment with closantel was 59.5% 6 weeks after inoculation. Exp. 2: all the treated groups had significantly less flukes compared to controls, with efficacies of 92 to 99%. Treatment with closantel 6 weeks after inoculation was 92% effective. **Conclusions:** Exp. 1 showed that triclabendazole at the recommended dose rate was ineffective against *Fasciola hepatica* aged 2 to 12 weeks, and at twice of the recommended dose rate the drug was ineffective against flukes aged 6 weeks. The failure to control the disease in the first property was due to the development of a total resistance to the parasite, possibly due to frequent treatments with the drug in a highly contaminated area. Treatment with closantel showed a moderate level of resistance against 6 week-old flukes. In Exp. 2 treatments at lower than the recommended dose rate showed high efficacy against the flukes aged 4 to 12 weeks. Closantel was also highly effective against that isolate. It has been shown that the development of serious resistance can develop to triclabendazole in highly contaminated pastures. Frequent treatments with the drug should be avoided. Strategic control, according to climate, should also include annual drug rotation.

From susceptible to resistant: levamisole selection in *Haemonchus contortus* in lambs.

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Starting with a susceptible population of *Haemonchus contortus* we were able to reach a high level of resistance to levamisole (LEV) by *in vivo*-selection in lambs within 6 generations. The susceptible population originated from Zimbabwe and probably had never been in contact with anthelmintics. In the first selection step, a lamb was infected with 5000 larvae and treated with 0.5 mg kg⁻¹ LEV after 3 weeks (3w p.i.). The egg output was reduced with 91.9%. One week after the first treatment the lamb was treated again with 1.0 mg kg⁻¹ LEV. The reduction was now 99.3%. Large amounts of faeces were collected and larvae cultured for the next infection. This lamb was dosed with 2 mg kg⁻¹ (3w p.i.). In the next selection step, a lamb was infected and treated with 4 mg kg⁻¹ (3w p.i.) and 5 mg kg⁻¹ (4w p.i.). The fourth lamb was treated with 7.5 mg kg⁻¹ (=therapeutic dose) (3w p.i.) and 10 mg kg⁻¹ (4w p.i.). The next lamb was dosed with 15 mg kg⁻¹ (3w p.i.) and 20 mg kg⁻¹. In the last selection step, a lamb was treated with 25 mg kg⁻¹ (3w p.i.) and 30 mg kg⁻¹ (4w p.i.). Selection was stopped because of the risk for sheep of toxic effects of higher doses. The selection process was reflected in the results of a newly developed egg hatch assay which was carried out in each lamb before treatment. A controlled trial was performed with susceptible larvae and larvae cultured from worms which survived 30 mg kg⁻¹. Six lambs were infected with the susceptible population and 6 with the resistant one. After 3 weeks, 3 lambs of each group were treated: the susceptibles with 7.5 mg kg⁻¹ LEV, the resistant ones with 30 mg kg⁻¹. The treatment of susceptibles was 100% effective, the treatment of the resistant lambs resulted in a reduction in worm numbers of 34%. It was remarkable that only the number of female worms was reduced. The selected population had a longer prepatent period, eggs had a longer development time, but egg production per female was not different compared with the susceptible one.

An experimental infection of a lynx (*Felis lynx*) with *Sarcoptes scabiei* var *vulpes*

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A lynx (*Felis lynx*) was experimentally infected with *Sarcoptes scabiei* from a naturally infected red fox (*Vulpes vulpes*) and the infection was studied clinically, histologically and serologically. For 20 weeks following infection, the lynx was observed daily for one hour at noon from a place 10 m from the cage. Skin scrapings and skin biopsies were taken once a month. An indirect ELISA, employing as secondary antibody a monoclonal anti-cat IgG, was used on sera prepared from blood samples taken once a week from the lynx.

Two weeks post infection (wpi) the fur on the back, where the infection had been applied was shortened and the skin was dry and discoloured. One week later the skin lesion had increased with alopecia, crusts and dandruff appearing. The skin lesion spread gradually, posteriorly around 25 cm on the back and dorso-cranially onto the head and the base of the ears. By 8 wpi the skin of the exposed area was partly hyperkeratotic. The animal was never observed to be scratching. At 20 wpi the lynx started to mutilate its tail and was therefore euthanized. At this time, patches of alopecia were also seen below the carpus and laterally on the elbow of a frontleg, and dorsally on the paw of a backleg. In addition erosions were evident medially above the elbow.

Skin biopsies at 4-6 wpi showed epidermal acanthosis, multifocal parakeratosis, serocellular crusts and subcorneal blisters containing mites. In the upper dermis moderate amounts of perivascular infiltrates of eosinophils, lymphoid cells and mast cells were found. In biopsies taken later, acanthosis, hyper- and parakeratosis were more marked. Otherwise changes were similar. Large numbers of Sarcoptic mites were seen in the subcorneal blisters and in skin scrapings. Serum antibodies to *S. scabiei* were demonstrated and seroconversion was seen at wpi 9-10.

031

Cyathostome burdens in Arab and mixed blood horses kept in identical husbandry conditions

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In order to study racial differences in parasite burdens in horses, the level of cyathostome infection was studied in a horse farm where pure Arab and sport type Argentine Saddle horses (ASH) are bred. The latter is an open lineage including thoroughbred, Argentine and European bloods. An outbreak of larval cyathostomiasis occurred in this farm 12 years ago. Following this, regular parasite drug control measures were started. Even so, 2/6 Arab and 1/44 ASH yearlings died of larval cyathostomiasis in the following years, when this control was relaxed. During our 30 months study, Arabs and ASH's lived together in the same large pasture lots, and parasite control and all other environmental and husbandry factors were identical for both breeds. To date, the farm is chronically infected with cyathostomes, but not with large strongyles.

Parasite loads were studied by egg per gram (epg) counts and examination of feces for adult parasites shed after drug treatments. Arabs in general had higher parasite loads than ASH's, and showed less age related reduction of these loads. (Mean epg's in adult horses: Arabs = 1147; ASH = 771; $p < 0.05$; mean epg's in horses aged 6-24 months: Arabs = 2253; ASH = 2561; p : ns). When individuals of both breeds were paired for identical sex, age and management conditions, young Arabs had similar burdens than young ASH's, but adult Arabs had larger ($p < 0.01$) burdens than adult ASH's. Five times, Arabs spontaneously shed cyathostomes between treatments; ASH never did. These data point to an altered host/parasite relationship.

030

Novel *S. typhimurium* aroA- vector: Targetted delivery of nematode parasite antigens to the sheep immune system

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Live, attenuated bacterial carriers such as aro mutants of *Salmonella* (deficient in the aromatic amino-acid biosynthetic pathway) are currently being developed world-wide as delivery vectors to target antigens from bacterial, viral and parasite pathogens to the host immune system.

We have developed two novel genetic tools (i) synthetic *in-vivo* inducible gene expression promoters (patent pending) and (ii) a site-specific chromosomal integration system, to integrate foreign antigen gene cassettes into the chromosome of *Salmonella typhimurium* aroA-strain (patent pending).

A *T. colubriformis* (gastrointestinal nematode parasite of sheep) extracellular/secretory antigen gene was cloned and expressed under the control of our *in-vivo* inducible promoters and the gene cassette was integrated into a non-essential site in the *S. typhimurium* aroA-chromosome. Recombinant salmonellae were demonstrated to stably express the *T. colubriformis* antigen and the gene expression promoters were demonstrated to be induced to high levels under *in-vitro* simulated *in-vivo* conditions.

The novel genetic tools provide a means to construct stable and optimal expression systems for foreign antigen delivery to the sheep immune system, via live attenuated Gram-negative bacterial carriers such as *S. typhimurium* aroA-.

032

Seasonal variation of *Amblyomma hebraeum* adult ticks collected by trapping in a communal grazing area in South Africa

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Amblyomma hebraeum is the main vector of heartwater in South Africa. Intensive dipping was, until recently, the control method of choice, but many policy makers are now advocating reduced strategic dipping of livestock. This has led to the recent spread of heartwater in Zimbabwe. A better understanding of the epidemiology of this important disease is necessary.

A. hebraeum adults do not "quest" for hosts, and are seldom picked up with larval flagging methods. They are, however, attracted to the "Attraction-Aggregation-Attachment pheromone" (AAP)/CO₂ trap.

The Rietgat communal grazing area in North West Province, was visited regularly from February 1996 to February 1997, and adult *A. hebraeum* ticks were collected with the AAP/CO₂ trap from five different ecological areas known to have ticks.

Adult *A. hebraeum* (n = 839), (males = 405, females = 434), were only collected from August through to December, after which the numbers declined rapidly.

At Rietgat, there is a short season from August to December, when conditions are optimal for the capture of wild, adult *A. hebraeum*.

Prevalence of *Cowdria ruminantium* in adult *Amblyomma hebraeum* ticks collected from a communal grazing area in South Africa

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Amblyomma hebraeum is the main vector of *Cowdria ruminantium* infection (heartwater) in South Africa. Although there have been several studies on the prevalence of *C. ruminantium* in *A. hebraeum* ticks, few have utilised large samples of ticks and a reliable diagnostic test. Additionally, no studies on infection rates have been conducted within communal farming systems.

Here, we describe the application of a polymerase chain reaction-based assay specific for *C. ruminantium* to provide estimates of the vector infection rate in *A. hebraeum* ticks from a communal grazing area.

One hundred and eighty unfed adult *A. hebraeum* ticks were collected using an attraction-aggregation-attachment pheromone/CO₂ trap at Rietgat, a communal farming region in the North West Province of South Africa. One hundred and fifty of the ticks were individually dissected and analyzed with the PCS20 P.C.R. assay for *C. ruminantium*. The remaining 30 ticks were fed on a heartwater-naïve sheep to determine if the ticks were infected with *C. ruminantium*.

Seven of 150 ticks were positive for *C. ruminantium* infection by P.C.R., giving a prevalence of 4.7%. The sheep developed heartwater and *C. ruminantium* was isolated from its plasma during the febrile reaction, confirming the presence of infection in the ticks.

This study confirms that *C. ruminantium* infection is present in the Rietgat communal farming area. The tick infection rate determined here falls within the range determined in earlier studies for *A. hebraeum* in commercial farming systems in South Africa.

035

The duration of protective efficacy of doramectin pour-on against *Linognathus vituli* infestation of cattle

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A study was conducted to determine the duration of protective efficacy of a single application of the pour-on formulation of doramectin at its recommended dose of 500 µg doramectin/kg against experimentally induced *Linognathus vituli* infestation of cattle.

Nineteen louse-free, male, Ayrshire calves with predominantly white hair were selected. On study day 0, eight of these animals chosen at random were treated topically with doramectin. The other eleven animals remained untreated and were used as controls to confirm infectivity of challenge infestations. Animals were individually penned for the duration of the study. Treated animals were subdivided into two equal groups (A and B) and commencing on day 14 with group A and day 21 with group B, groups were challenged on alternate weeks by placing 50 adult lice freshly collected from donor calves on the withers of each calf. The final challenge was on day 84 (group A). Control calves were each similarly challenged on one occasion; four on day 7, two on day 21 and one on each of days 35, 49, 63, 71 and 84. Louse and louse egg counts were performed on designated predilection sites for each animal at weekly intervals from initial challenge.

Progressive louse infestations established on all control calves confirming infectivity of challenge infestations. Live lice infestations first established in treated animals following the day 77 challenge in group B and following the day 84 challenge in group A. Infestations were present in only one animal in each group. Overall, the latest challenge that failed to establish was applied on day 71, hence it can be concluded from this study that a single topical application of doramectin at a dose rate of 500 µg/kg protects cattle against *L. vituli* infestation for at least 71 days.

Seasonal variation of *Amblyomma hebraeum* ticks collected off cattle and goats on two communal grazing areas in the North West Province of South Africa

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Amblyomma hebraeum is the main transmitter of heartwater in South Africa, and although the population dynamics of this important tick are well documented, there are few reports on the tick levels from domestic animals on communal grazing areas.

Ticks were collected off cattle (n = 3), and goats (n = 3), on a monthly basis from September 1991 to August 1993. The two different communal grazing areas which were visited on a regular basis were Rietgat and Madinyane.

The cattle were cast with ropes and 13 different anatomical sites were individually sampled. The goats were restrained on the kraal floor and similar anatomical areas were sampled.

A. hebraeum adults were the most common tick on the cattle at Rietgat, and were present throughout the year. As many as 70 adult *A. hebraeum* were found per bovine. Nymphal *A. hebraeum* were also common on the goats throughout the year.

A. hebraeum was found virtually throughout the year on cattle and goats on the communal grazing areas of Rietgat and Madinyane.

This should have important implications for the endemic stability of heartwater, as both the cattle and the goats will be exposed to *Cowdria ruminantium* throughout the year.

036

Persistent efficacy of doramectin and ivermectin against naturally acquired *Boophilus microplus* infestations of cattle in Latin America

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Multicenter studies were conducted in Venezuela, Brazil, and Colombia to compare the persistent efficacy of doramectin and ivermectin against naturally acquired *Boophilus microplus* infestations in cattle. After enumeration of engorged female ticks on the right side of each animal and random allocation of cattle to 2 treatment groups according to a block design, cattle (9-120 months old) were treated with doramectin or ivermectin (200 µg/kg, SC) on day 0 of the study. All studies followed the same experimental protocol but varying the post-treatment (p.t.) observation days in each country. In Venezuela, the study included 810 cattle on 27 farms (30 cattle/farm). Tick counts were made on days 12 and 32 p.t. The Brazilian study involved 1,680 cattle on 56 farms (30 cattle/farm). Subsequent tick counts were conducted on days 12 and 28 p.t. In Colombia, 1,848 cattle on 43 farms (30 to 68 cattle/farm) were used. Post-treatment tick counts were on days 14 and 35. Cattle of each farm were maintained together in their original pastures throughout the study.

In all countries, doramectin was more efficacious than ivermectin in removing *B. microplus* infestations. In Venezuela, the overall mean efficacy of doramectin was 97.3% on day 12 p.t. and 71.9% on day 32 p.t., compared with 89.5% and 25.4% for ivermectin on days 12 and 32 p.t., respectively. On day 32 p.t., doramectin had ≥ 90% efficacy on 19 of 27 farms, whereas ivermectin achieved this efficacy on only 6. In Brazil, the overall mean efficacy of doramectin was 94.1% on day 12 p.t. and 92.8% on day 28 p.t., compared with respective values of 83.4% and 63.8% for ivermectin. From the 56 replicates, doramectin had 46 results with efficacy ≥ 90% on day 28; ivermectin had similar efficacy for only 21 replicates. In Colombia, the overall mean efficacy of doramectin was 99.2% on day 14 p.t. and 92.0% on day 35 p.t., compared with 96.1% and 65.1%, respectively, for ivermectin. At day 35, doramectin treatment resulted in efficacy ≥ 90% on 31 of the 43 farms, whereas ivermectin had similar results on only 12.

By the end of the study, the mean efficacy of doramectin in removing *B. microplus* infestations (88.1%) was significantly ($P < 0.0001$) higher than that of ivermectin (56.2%), when data from all countries was combined.



Persistent efficacy of doramectin and ivermectin for prevention of naturally acquired *Cochliomyia hominivorax* infestation of cattle in Latin America

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Multicenter studies were conducted in Argentina, Brazil, Colombia, and Venezuela to compare the persistent efficacy of doramectin and ivermectin for prevention of myiasis caused by *Cochliomyia hominivorax* larvae in cattle castrated 10 days after treatment and exposed to field challenge. The studies included 135 replications (7 farms in Argentina, 91 in Brazil, 13 in Colombia, and 24 in Venezuela) and involved 4,148 cross-breed cattle. For each trial, 26 to 60 male cattle were selected and randomly allocated to 2 groups of equal size. On day 0, cattle were treated with doramectin or ivermectin (200 µg/kg, SC). On day 10 post-treatment (p.t.), all animals were castrated surgically according to farm practices, and cattle of both treatment groups were maintained together in the same pastures on each farm. On days 13 and 17 p.t. (3 and 7 days after castration), cattle were examined, and the presence or absence of oviposition, active myiasis and status of healing were evaluated. Active myiasis was defined by the presence of larvae, hemorrhage, and exudate.

In all countries, doramectin was more efficacious than ivermectin in the protection of castration wounds against *C. hominivorax* infestation. In Argentina, the overall efficacy of doramectin was 100% in all 7 trials; ivermectin had an overall efficacy of 80.4% with efficacy ≥ 90% in only 2 trials. In Brazil, the overall efficacy of doramectin was 94.6%, compared with 43.7% for ivermectin. Doramectin treatment resulted in efficacy ≥ 90% in 73 of 91 trials, whereas ivermectin treatment provided ≥ 90% efficacy in only 3 trials. In Colombia, the overall efficacy of doramectin was 97.2%, compared with 55.0% for ivermectin. Efficacy was ≥ 90% in 12 of 13 trials for doramectin, but in only 1 of 13 trials for ivermectin. In Venezuela, the overall efficacy of doramectin was 95.2%, compared with 52.2% for ivermectin. Efficacy ≥ 90% was determined in 20 of 24 trials for cattle treated with doramectin, whereas ivermectin had ≥ 90% efficacy in only 4 trials. When data from all 4 countries was combined, the mean efficacy of doramectin in preventing *C. hominivorax* infestation of castration wounds in cattle (95.2%) was significantly ($P \leq 0.0001$) higher than that of ivermectin (48.2%).

Practical application of an *in vitro* larval development assay to detect anthelmintic resistance in sheep nematodes in South Australia

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Drenchrite® is an *in vitro* larval development assay (LDA) for the detection of resistance to benzimidazole (BZ), levamisole (LV), BZ/LV combination (CM) and avermectin/milbemycin (ML) drenches in *Haemonchus*, *Trichostrongylus* and *Ostertagia*. Nematode eggs are hatched in the wells of a microtitre plate and larvae develop to the third larval stage in the presence of anthelmintic. The concentration of drug required to block development is related to an anticipated *in vivo* efficacy, which can be quantified with all drench groups except ML.

The drench resistance status of 101 South Australian (SA) sheep properties was examined using the LDA. Overall prevalences of resistance (anticipated *in vivo* efficacies of < 95 %) were 95 %, 72 %, 37 % and 0 % for BZ, LV, CM and ML drenches respectively. These estimates are comparable to those determined by others from 118 SA properties (93 %, 72 %, 45 % and 0 % respectively) using the conventional faecal egg count reduction test.

The LDA indicated a prevalence of resistance for *Trichostrongylus* against BZ, LV and CM drenches of 99 %, 88 % and 54 % respectively compared with 79 %, 38 % and 20 % for *Ostertagia*. This is alarming because *Trich. spp.* are the most important worms of sheep in SA. The plate position at which ML exposure reduced *Trich.* development by 50 % shifted 1 - 2 wells beyond the specified susceptible range (ssr) in 20 % of assays from widespread locations. This event was unrelated to the frequency of exposure to ML drenches, drench failure was not reported and faecal egg counts were reduced 100 % by drenching with ML, hence resistance is not suspected. The "movement" is attributed to differences between the 3 *Trich.* species common in SA and the susceptible isolate of *T. colubriformis* used to standardise the plate ranges.

"Movement" of a dominant *Ostertagia* population to 1 well beyond the ML ssr occurred on 1 property. Small numbers of larvae from non-dominant *Ost.* populations on 2 properties in the same area survived 1[] wells beyond the ssr. All properties have been using ML drenches recently. These LDA findings may provide the first, highly sensitive evidence of field emergence of ML resistance in *Ost.* populations in SA.

*Trademark of Horizon Technology Pty Limited, Roseville, NSW, Australia.

Helminth parasitism in alpacas in southern Australia

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The foundation stock of Australia's rapidly developing alpaca industry mainly comprises direct imports from Chile and Peru plus small numbers via New Zealand. All alpacas are treated for worms at least 3 times over a 100-180 day period during the import process, usually with ivermectin by injection. Their great commercial value has determined that, after arrival, regular similar treatments have continued, without consideration for their need or long term outcomes.

The helminth levels in southern Australian alpacas were examined on 5 farms over 2 years. Faecal samples were collected from at least 20 alpacas of various age classes on 10 occasions, faecal egg counts were estimated, nematode larvae identified from faecal cultures, and cultured larvae dosed to worm-free lambs for recovery and speciation of adult worms.

Lamoid-specific helminths were not found and have not yet been reported from alpacas in Australia, possibly because their introduction or establishment has been limited or prevented by repeated treatments with macrolides. Common cattle and sheep helminths, including *Ostertagia circumcincta*, *Trichostrongylus colubriformis*, *T. vitrinus*, *Cooperia oncophora*, *Oesophagostomum venulosum* and *Chabertia ovina* were recovered following transmission of larvae derived from alpacas to sheep. *Haemonchus contortus*, *Dicrocoelium filaria* and *Fasciola hepatica* were not found in this study but are potentially dangerous for alpacas.

From June-August, which is the period of major pasture larval availability, crias (< 6 months) and previously unexposed weaners (6 months-1 year) developed peak faecal egg counts as high as 1300 epg which declined naturally to low levels within a few months. Neither clinical nor subclinical helminthosis were suspected on any of the farms. Tuis (1-2 years) and adults (> 2 years) which were untreated for worms for over 2 years did not develop appreciable faecal egg counts. Alpacas grazing sympatrically with sheep had a lower worm egg output than the sheep, suggesting that they have a greater inherent resistance to sheep worms. It is recommended that a monitoring system involving "at risk" animals is instituted for helminth control and that, rather than herd treatments, those with elevated faecal egg counts are treated individually. This will reduce selection pressure for anthelmintic resistance, especially against cattle worms carried by alpacas.

Comparative efficacy of program use of two doses of doramectin and ivermectin in the control of endo- and ectoparasite infestations and their effects on productivity of fattening cattle

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A study was conducted in Malacacheta, Minas Gerais State, Brazil, to compare the efficacy of the program use of 2 doses of doramectin with that of 2 doses of ivermectin in the treatment and control of endo- and ectoparasites and their effects on the productivity of fattening cattle for a period of 140 days. Criteria for comparison were tick counts, tropical warble nodule counts, nematode eggs per gram (EPG) of feces, and weight gain.

Sixty cross-breed Zebu by Holstein noncastrated males, with ages ranging from 18 to 20 months, were randomly allocated to 2 groups (T1 and T2) of 30 animals each, based on body weights. Animals of T1 were treated on Days 0 and 55 by subcutaneous injection of doramectin at a dose rate of 200 µg/kg. Animals of T2 were treated on those same days with ivermectin at a dose rate of 200 µg/kg. Animals were maintained in 2 equal but separate pastures throughout the experimental period of 140 days. On Days 0, 28, 55, 84, 112 and 140, animals were individually weighed and parasite burdens evaluated. Fully engorged female *Boophilus microplus* were counted on the right side of each animal; tropical warble *Dermatobia hominis* nodules were counted on all body surfaces. On those same days and additionally on Days 41 and 97, fecal samples were collected for EPG determination. One animal of group T2 (ivermectin-treated) died of unrelated causes after the had started and, thus, this group consisted of only 29 animals.

Doramectin was significantly more efficacious ($P < 0.05$) than ivermectin in the treatment and control of ticks and in reducing nematode EPG counts. *D. hominis* nodule counts were too low for meaningful analysis. The mean weight gain of cattle treated with doramectin was 39.7 kg, while ivermectin-treated animals had a mean weight gain of 31.5 kg. This difference was statistically significant ($P < 0.05$).

Periparturient rise in faecal egg counts associated with prolactin concentration increase in French Alpine dairy goats.

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Previous data about periparturient relaxation of immunity in gastrointestinal nematode infection in goats are scarce and conflicting: one study realized in fiber (Angora) goats showed the association of faecal egg counts and prolactin concentrations around parturition whereas the two other available studies dealing with dairy goats gave divergent results. Twenty eight French Alpine grazing dairy goats, naturally infected with *Teladorsagia*, *Trichostrongylus* and *Oesophagostomum*, were allocated in two groups according to their reproductive status: group 1 (n=7) consisted in non-pregnant lactating animals in the 3rd month of lactation whereas group 2 (n=21) was composed of dry goats 6 weeks before term. Faecal egg counts, pepsinogen and phosphate concentrations, blood eosinophil counts and prolactin concentrations were individually monitored for 12 weeks (mid winter to early spring).

The mean faecal egg counts were significantly higher in pregnant goats during the two weeks before (668 vs 242 epg, P<0.05) and the two weeks after (962 vs 279 epg, P<0.01) the parturition compared to non-pregnant lactating animals. *Oesophagostomum* infective larvae were predominant in coprocultures particularly at the time of parturition. Nevertheless, no significant difference was seen in composition of larval culture between the two groups of animals. Pepsinogen and phosphate concentrations as well as blood eosinophil counts were similar between the two groups throughout the survey and indicated a moderate larval challenge. The mean prolactin concentration of pregnant goats was significantly higher (P<0.01) at the time of parturition (298 vs 130 ng.ml⁻¹) and 4 weeks after parturition (387 vs 193 ng.ml⁻¹) than that of non-pregnant animals. Furthermore, a significant correlation (r=0.30, P<0.01) between faecal egg count and prolactin concentration was recorded for pregnant goats 4 weeks around parturition.

Efficacy and pharmacokinetics of levamisole hydrochloride in experimental infections with *Haemonchus contortus*, *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* in dairy goats

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As benzimidazole resistance is being highly prevalent in goat nematodes, levamisole hydrochloride could represent a valuable alternative for controlling nematode during the milking period. In order to avoid signs of toxicity in relation with plasma concentration peak, two administrations of levamisole (12 mg.kg⁻¹ BW) were compared, single vs repeated as two half-doses 10 hours apart, both in terms of efficacy and pharmacokinetics.

Fifteen young female goats, 3 months old, were inoculated with 2000 *T. colubriformis* third stage larvae (L3) on day -1, 1000 *H. contortus* L3 and 2000 *T. circumcincta* L3 on day 0. On day 25 post-infection, goats were randomized into an untreated control group and the two levamisole treatment groups (1 x 12 or 2 x 6 mg.kg⁻¹ BW). Blood samples were collected before and sequentially after each administration. Quantification of levamisole was realized by HPLC. The goats were killed and processed for worm recovery 10 days after treatment.

No adverse reactions were seen in young goats as in lactating adult ones following the two types of levamisole treatment. Both dose rates achieved similar levels of efficacy against *H. contortus* (> 99 %). Against *T. circumcincta* and *T. colubriformis*, the efficacy of the repeated administrations was slightly lower than the single one (respectively 91.7 vs 96.2 and 92.5 vs 100) although the difference was not significant. Following an oral dose of 12 mg.kg⁻¹, the Cmax Value (1.38 µg.ml⁻¹) obtained at the first sampling time (10 min) reflected fast mechanism of absorption. After two half-doses (2 x 6 mg.kg⁻¹) the Cmax Values were 0.93 ± 0.47 µg.ml⁻¹ for the first administration and 1.43 ± 0.37 for the second administration. Both dose rates achieved similar areas under the curve (AUC) respectively 3374 ± 1565 ng.h.ml⁻¹ for the single dose and 4426 ± 775 ng.h.ml⁻¹ for the repeated administration and indicated equivalent bioavailability of the treatments.

Development of resistance to *Trichostrongylus colubriformis* in goats: influence of an initial trickle infection abbreviated or not on the response to a subsequent challenge.

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In order to investigate the effect of a trickle infection on the response of goats to a nematode challenge infection, 18 non lactating and non pregnant, 2 to 6 year-old, French Alpine goats were allocated into 3 groups of 6 animals. Goats were naive regarding strongyle infection. Groups 1 and 2 received weekly, for 10 weeks, 7,500 infective larvae (L3) of a benzimidazole (BZ)-susceptible strain of *T. colubriformis*. Meanwhile, animals in group 3 remained uninfected. On week 10, goats in group 2 were given fenbendazole (FBZ, 10 mg.kg⁻¹) to remove the pre-existing worm burdens and on week 11, all the goats received a challenge infection composed of 20,000 L3 of a BZ-resistant strain of *T. colubriformis*. Three weeks post-challenge, the animals were treated with FBZ to remove the susceptible worm population. Six days after their last treatment, the goats were necropsied to assess the establishment of the challenge infection.

Mean intestinal worm burdens were considerably lower in goats of groups 1 and 2 (respectively 24 and 59) compared to those of group 3 (641). The other parasitological data related to the expression of host resistance which were investigated were nematode fecundity (number of eggs per female) and % of male. Both were significantly lower in group 1 compared to group 3 whereas group 2 showed intermediate values. Goats of group 1 had higher values for blood and intestinal tissue eosinophil numbers compared to animals of groups 2 and 3. In contrast, goats of group 2 had higher values in intestinal globule leucocyte numbers. No significant difference in intestinal mast cell numbers was seen between the 3 groups. Considering all the animals, intestinal worm burdens appeared to be significantly correlated with intestinal eosinophil and globule leucocyte counts (-0.51 and -0.49 respectively, df=16).

These results showed that goats were able to develop a high level of resistance to *T. colubriformis* infection after a trickle infection. In the condition of our experiment, removing the existing worm burden prior to challenge led to a lower extent of the host response both in terms of nematode fecundity and eosinophil populations.

Interaction of *Verticillium Chlamydosporium* with nematode ova

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The hyphae of *Verticillium chlamydosporium* enveloped ova of *Ascaridia galli* and *Parascaris equorum* on water agar and progressively eroded the eggshell. Subsequently, after penetration of the infection hyphae of *V. chlamydosporium* into the ova, the zygote and/or developing embryos were destroyed by hyphal growth within three days for *A. galli*, but complete destruction of *P. equorum* ova took six to twelve days. Although the ova of *Trichuris suis* were often contacted by hyphae of *V. chlamydosporium* on water agar, the ova were rarely invaded by the hyphae. These findings suggest that *V. chlamydosporium* would be useful in interruption of the life cycle of ascarids. However, the rare interaction of *V. chlamydosporium* with *T. suis* suggests that adenophorean ova may have very low levels of attractive factors or receptors for this fungal parasite.

Field evaluation of Ivomec-F for treatment of naturally acquired *Fasciola gigantica* and other gastrointestinal nematode infections in cattle and buffalo

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The efficacy of Ivomec-F was evaluated in 60 cattle and 14 buffalo that had been naturally acquired infection with *Fasciola gigantica* and some were concurrent infected with gastrointestinal nematodes. The animals were equally divided into treated and control groups: group 1, 30 treated cattle were subcutaneously administered a single dose of Ivomec-F (1 % ivermectin / 10 % clorsulon) 1ml/50kg body weight; group 2, 30 untreated control cattle were physiological saline administered at the same dose; group 3 and group 4 (7 treated and 7 control buffalo) were performed at the similar manner as in cattle. Fourteen days after treatment, 5 animals from each group of cattle and 3 animals from each group of buffalo were slaughtered and examined for parasites in bile ducts, gall bladder, liver parenchyma and gastrointestinal tract. The faecal and blood samples from all animals were collected at day 0 before treatment and weeks 2, 4, 8, 12, 16, 20 and 24 after treatment. Faecal examination were determined for *Fasciola* eggs count per gram (epg) by the beads technique and the presence of gastrointestinal nematode eggs by brine floatation. Standard haematological tests (total rbc, total wbc, Hb, Ht, MCV, MCH and MCHC) and selected biochemical parameters (total protein, albumin, iron, creatinine, BUN, GGT, AST and ALT) were measured. The results showed that no living flukes in bile ducts, gall bladder and liver parenchyma or nematodes in the gut were remained in the treated groups of slaughtered animals. Only few eggs of liver fluke were found in the bile of 2 treated cattle in one buffalo. By the mean time, neither eggs of liver fluke nor gastrointestinal nematodes were found in faeces of all treated animals from week 2 to 16 after treatment. This study was clearly shown that Ivomec-F was significantly highly effective against mature *F. gigantica* and gastrointestinal nematodes in cattle and buffalo. In addition, there was no effect of Ivomec-F on any haematological and biochemical parameters.

Echinococcus proliferation and protoscolex histogenesis

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Proliferation of echinococcus and histogenesis of protoscolex including CE (cystic echinococcosis) and AE (alveolar echinococcosis) were morphologically observed by means of histopathology or imaging examinations (ultrasound and CT). In spite of CE or AC, the proliferative mode shared endogenous and exogenous budding, and some new phenomena were discovered. CE exogenons budding may be expressed as externally protrusive proliferation from local wall of a mother cyst, or migration of protoscolex from the hydatid cavity to laminated layer and then to the extracystic tissues. CE endogenons budding showed daughter and granddaughter cysts from local hyperplasia of germinal membrane. As to AE, exogenons budding may always produce single or multiple daughter or granddaughter alveoli. AE endogenous budding was characterized by internally protrusive proliferation from mother alveolar wall into the alveolar cavity and extended continuously to the opposite wall, thus forming a septum to divide the mother alveolus into two or several alveoli. The process of protoscolex histogenesis was described in detail and it must need to pass through a stage of the formation of brood capsule (BC). But the mode of BC formation in AE and CE was somewhat different due to the possible differences of parasitic species or strains.

Effects of different *Oesophagostomum dentatum* population densities following single or trickle infections in pigs.

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Oesophagostomum spp infections are widespread in pigs throughout the world. The population biology and dynamics of one of the common nodular worm species in pigs, *Oesophagostomum dentatum*, is, however, only known to a limited extent. Two recent studies have examined the effect of different population densities of *O. dentatum* in pigs. Following a single infection at different dose levels to three groups of pigs (each pig received 2,000, 20,000, or 200,000 infective third stage larvae (L₃)), a negative correlation was found between the inoculation dose and the onset and level of egg excretion. Additionally, the fecundity of the female worms (egg per female worm) was significantly reduced in the high dose group. Subgroups of the pigs were slaughtered at frequent intervals after infection (p.i.). In the high dose group, the worm development was significantly retarded and the adult worms were significantly smaller when compared to the other groups. Furthermore, the proportion of recovered worms was significantly lower in the high dose group at the end of the experiment (on day 42 p.i.). It was obvious from this study that high population densities exerted a negative impact on the overall population. To obtain better knowledge of the population dynamics of *O. dentatum*, a trickle infection experiment was undertaken where 3 groups of pigs were inoculated with *O. dentatum* via the feed twice weekly with 100, 1,000, or 10,000 L₃. Subgroups of pigs were slaughtered 2, 4, 8, 12, 16, and 20 weeks after the first inoculation to determine their worm burdens. Faecal egg counts were proportional to dose rate until week 15, but later were more variable. Mean worm recovery was inversely proportional to the dose rate, but in the high dose group decreased over time, whereas in the 2 other groups there was no consistent pattern over time. Worm fecundities in the two lower dose groups were similar to each other and greater than in the high dose group. The lengths of the female worms increased over time, whereas the lengths of the male worms remained approximately constant from week 8 p.i. This study suggested a reduced establishment of incoming larvae and lower fecundity of the female worms at the high dose level.

Cystic Echinococcosis of Yak in China

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Yak (*Bus grunniens*) which covers over one-sevenths of all the cattle in China is a specialized species of the livestock in Qinghai - Xizang Plateau, and it only distributes in the high - cold regions of five provinces or autonomous regions including Gansu, Qinghai, Zinjiang, Tibet and Sichuan. According to an incomplete statistics in China 2 years ago, there were about 12.3 millions of the yak which accounted for 85 % of the total yak all round the world. The author investigated that during a period of 7 years (1985-1992) 57947 yak were slaughtered with an average of 8278.1 per year in Ganan Prefectural Meat - Packing Plant, Gansu. The annually average infection rate of the organs was 3.96 %, 5.27 % and 0.12 % respectively in liver, lung and heart. The visceral distribution of yak echinococcosis was more frequent in the lung than in the liver, while the location of sheep echinococcosis was on the contrary. From the Chinese literatures, the infection rate of yak echinococcosis in the other four provinces were as follows: 30.71-74.7 % in Qinghai according in the reports of 4 series between 1980 and 1989; 5.2 % (19/365) - 81.3 % (26/32) in two counties and one city of Zinjiang; 49.9 % (1820/3645) in the borderland between Sishuan and Tibet according to a report in 1988. As mentioned above, the infection rate of yak echinococcosis varied with different endemic areas.

Interactions between preventive anthelmintic treatment and acquired resistance to gastrointestinal nematodes in artificially infected cattle.

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The objective of this study was to evaluate the effects of different intensities of chemoprophylaxis given during a simulated "first grazing season" on the resistance of calves to an artificial challenge infection with *Ostertagia ostertagi* and *Cooperia oncophora*. Thirty six 7-11 months old Holstein-Friesian bull calves were randomly divided in 4 groups. The animals of group B received an ivermectin sustained release bolus on day 0. The calves of group D were treated on days 0 and 56 with a subcutaneous injection of doramectin (0.2 mg/kg BW). Group C was the untreated control group. The calves of group N were used as helminth-naïve controls, while the animals of groups B, C and D were trickle infected for 24 weeks. The infection schedules were designed to simulate the expected infection pattern for each treatment group under set-stocked conditions in temperate climate areas. After the last infection all animals were treated with oxfendazole. One week later all animals received a challenge infection of 50,000 *O. ostertagi* L3 and 100,000 *C. oncophora* L3, spread over 10 consecutive days. During the primary infection period the faecal egg output and the serum pepsinogen and antibody levels indicated different levels of host-parasite contact between the groups (group C > group D > group B > group N). After the challenge infection faecal egg counts, total *Ostertagia* burden, size of the adult worms and abomasal globule leucocyte counts indicated a positive relationship between the level of exposure to *Ostertagia* during the primary infection period (determined by the infection schedule and the chemoprophylaxis used) and the level of acquired resistance to the *Ostertagia* challenge infection. Faecal cultures and small intestinal worm counts indicated that all previously infected groups had acquired a high degree of resistance to the *Cooperia* challenge infection. In conclusion, a reduction of host-parasite contact during the primary infection period, as a consequence of the infection schedule and the chemoprophylaxis used, resulted in a reduced level of resistance to a high artificial challenge infection with *O. ostertagi*.

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Assessment of the persistent activity of IVOMEC Pour-On and doramectin injection against biting lice infestations in cattle

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From a group of stockyard-purchased cattle, 90 mixed breed heifers and steers were allocated on the basis of sex and bodyweight among 45 uniform pens, which were arranged in 15 blocks of three pens each. Construction was designed to prevent cross contamination between pens. A short-acting organophosphate spray was applied to all cattle 14 and 7 days prior to the start of the trial, to remove existing louse infestations. On Day 0, cattle within each pen block were allocated to three treatments: 1. untreated control; 2. doramectin by subcutaneous injection (minimum dose 200 µg/kg); 3. IVOMEC® (ivermectin) Pour-On, (minimum dose 500 µg/kg). On Day 21, 15 louse-infested (*Bovicola bovis*) donor calves were placed with trial cattle in the first 15 pens, one donor per pen, to provide a challenge to five pens of each treatment group. Donors remained in trial pens for 14 days. The challenge procedure was repeated for the second 15 pens on Day 28, then for the final 15 pens on Day 35. Louse counts were completed for all trial cattle 7, 14 and 21 days after challenge. At each sampling, nearly all control and all doramectin-treated cattle in each challenge period were infested with *B. bovis*. Cattle treated with ivermectin remained virtually free of infestation throughout the 56-day post-treatment period. The results indicate that cattle treated with IVOMEC Pour-On are refractory to challenge with *B. bovis* for at least 35 days after treatment.

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Immune suppression to adult *Rhipicephalus appendiculatus* feeding as a consequence of previous or simultaneous immature feeding

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The immatures of many of the two- and three-host species feed on the same host as the adults. Successful intra-specific cross-resistance would therefore, have an important impact on the performance of such species.

All three the instars of *Rhipicephalus appendiculatus* feed on the bovine host as prime host. The life-cycle is such that the peaks of activity of the instars correspond. The immune response elicited in these hosts would therefore be a consequence of the combined feeding of all three stages.

Using the rabbit as model, the development of an immune response was achieved by the successive release of flat ticks in bags glued to the backs of the hosts. In this manner the effect of an existing response on the feeding success of another instar could be determined.

Intra-specific cross-resistance exists between the three stages, but the presence of an anti-larval response seems to suppress the development of a complete anti-nymphal response as experienced in hosts subjected to nymphal feeding only. The mean weight of samples of five replete nymphs retrieved from a third nymphal infestation from larval sensitised hosts was 48.8 ± 7.67 mg, compared to a third infestation mean of 28.62 ± 5.27 mg preceded by two nymphal infestations only. Furthermore, the mean replete female weight from hosts previously exposed to a combination of immature feeding was 194.46 ± 145.99 mg compared to 46.55 ± 27.2; 99.4 ± 84.1 and 218.66 ± 150.64 mg after three infestations, respectively only with adults, nymphs and larvae. The adults actually seemed to feed under more favourable conditions in the presence of the combined immature immune response than in the presence of a response to either one of the immature stages.

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A microagar larval development test for anthelmintic resistance

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A microagar larval development test [MALDT] has been developed to detect resistance to benzimidazoles, levamisole and ivermectin in *Ostertagia circumcincta* and *Haemonchus contortus*. Ten ml of anthelmintic solutions, and 150ml of 2 % agar in water are added to a 96 well plate using multistep pipettes. Eggs are collected from faecal samples by centrifugation in saturated salt and washing twice in water. Equal volumes of eggs and solubilised amphotericin b (250mg/ml) are mixed and 10ml of stirred egg suspension added to each well with a single volume pipette to give 40 - 60 eggs per well. Ten ml of yeast extract [as prepared in Hubert and Kerboeuf (1984), but 2x concentrated] are added with a multistep pipette and the plate sealed and incubated at 25°C for 7 days. Since outer wells can give erratic results these are filled with water to prevent desiccation in other wells. After 7 days the numbers of third stage larvae are counted. Thiabendazole is used for benzimidazole resistance, levamisole for levamisole/morantel resistance, ivermectin monosaccharide for ivermectin resistance in *O. circumcincta* and avermectin B2 for ivermectin resistance in *H. contortus*. Other analogues, ivermectin, avermectin A1, A2, B1 and ivermectin aglycone were not satisfactory. Using this test the first British case of levamisole resistance associated with lamb deaths was confirmed and two cases of triple resistant *O. circumcincta* in Angora goats were detected, the first reported in the UK.

The genetics of anthelmintic resistance in *Ostertagia circumcincta* and *Haemonchus contortus*

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The genetics of anthelmintic resistance have been determined by making F1 and F2 (back) crosses of single or multiple resistant isolates of *O.circumcincta* and *H.contortus*. UK susceptible nematodes were crossed with either UK benzimidazole resistant worms or Australian levamisole resistant or UK triple resistant *O.circumcincta* or US levamisole-benzimidazole or South African benzimidazole-ivermectin resistant *H.contortus*. 12-14 day old worms were sexed and introduced into worm naive lambs following intravenous injection of lysine-vasopressin to cause oesophageal groove closure. Eggs shed were either analysed with the egg hatch or microagar larval development tests or cultured for infection of other lambs. In agreement with recent publications levamisole resistance was recessive in both species and ivermectin resistance was dominant in *H.contortus*. Tests for ivermectin resistance in *O.circumcincta* are in progress and will be described. Benzimidazole resistance was maternally inherited in the both species in the F1 generation, but in contrast to the published results with *H.contortus*, resistance was recessive in both species in the F2 generation. This difference may be explained by the extensive selection used in earlier work prior to the crosses which could have removed the genes for low level benzimidazole resistance.

Prevalence of *Gastrophilus* in UK equids

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Forty one adult horses, 4 brood mares, 4 foals and 13 Dartmoor ponies were examined at a licensed slaughter house in the south of England on 3 occasions during December 1995 and January 1996. *Gastrophilus* larvae were found in 63 % of animals with a mean of 35 larvae per animal. All animals which were known not to have received anthelmintic treatment recently were found to be infected. *Gastrophilus intestinalis* was present in all infected animals and was also found in the highest numbers, with a maximum of 197 larvae in one of the Dartmoor ponies. *Gastrophilus nasalis* was also present in 11/13 ponies and in one horse from the same area. A single *Gastrophilus pecorum* was found in one of the brood mares. The survey demonstrates that *G.nasalis* is prevalent in the southwest and that *G.intestinalis* remains common in the southern part of England.

Activity of moxidectin against bots and lungworms in equids

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Moxidectin is a milbemycin with high activity against a range of endo and ectoparasites in both farm animals and horses. The objectives of the present work was to determine its activity against natural infections of *Gastrophilus nasalis* and *Dictyocaulus arnfieldi*.

Eight naturally infected Dartmoor ponies acted as controls and 8 were treated with 0.4mg/kg paste. In early February 34 days after treatment ponies were humanely slaughtered and the numbers of bots, small and large strongyles counted. Moxidectin was 100 % effective against *G.nasalis*, 99.9 % against *G.intestinalis*, 100 % against *Strongylus vulgaris*, *Triodontophorus* sp and small strongyles and 99 % against *S.edentatus*. As would be expected in free ranging animals encysted larvae were present in very small numbers and were therefore not counted.

A herd of donkeys naturally infected with *D.arnfieldi* was identified in July and treated with moxidectin oral gel (0.4mg/kg). 3 ponies naturally infected with *D.arnfieldi* acted as controls, but two lost their infection by day 14. All 6 treated ponies had no larvae in their faeces by day 8 and remained negative at day 21. Moxidectin was 99.9 % effective against faecal nematode egg counts. The trials confirm and extend information on the high anthelmintic activity of moxidectin.

From research to commercial blood vaccine production, pitfalls

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From past experiences we have learnt that it is not easy to go from small scale controlled product research and development to large scale commercial production and application of the blood vaccines.

Large scale commercial production of a frozen blood vaccine is rather complex and involves at least 56 different steps. Although half of these lie outside the scope of the scientific research project only 13 or less were previously investigated. Based on this information and on acquired past experience, decisions were taken to commercially produce and supply vaccines. Invariably pitfalls were experienced.

The blood vaccine against heartwater was at one stage replaced by a tick derived vaccine. However, during the period in which it was widely used under field conditions, the occurrence of serious shock in lambs and especially goat kids (which was not revealed during initial experiment) led to its withdrawal and reinstatement of the blood vaccine.

Initial commercial production of small quantities of frozen anaplasmosis and babesiosis vaccines were done according to the research protocol and successfully frozen and stored in the gas phase of liquid nitrogen. An expiry time of 2 years were chosen considering the proven life expectancy of more than 10 years for blood stabilates. However, with large scale production, problems were experienced with the infectivity of *Babesia bigemina* after only 3 to 5 months storage.

Small scale laboratory controlled production, storage and testing of a freeze dried heartwater infective blood vaccine proved to be quite successful. When large scale production was investigated, problems were identified with freeze-drying of the vaccine in large freeze-dryers as well as with results obtained from infectivity testing of the vaccine in field trials.

It has since become policy to include all of the aspects concerning the large scale production of a vaccine into our initial research. In compliance with this requirement we are currently investigating the possibility of large scale production of *in vitro* grown *Cowdria ruminantium* organisms for use as a frozen heartwater vaccine and the replacement of the dimethyl sulfoxide cryoprotectant used in the frozen blood vaccines with a less toxic product such as glycerol.

Neosporosis: characterization of new isolates and application of newly developed molecular tests

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This presentation will focus on results obtained from the characterization of new bovine, equine and canine isolates of *Neospora*. Parasites isolated from an infected bovine fetus (BPA1) and congenitally infected calves (BPA3-7) have been cultivated *in vitro* and characterized. Antigenic and molecular comparison between these isolates from cattle and canine isolates indicates that they are most likely the same species. By contrast, a recent *Neospora* isolate from a horse with equine protozoal myeloencephalitis (EPM) showed consistent antigenic and molecular differences from the bovine and canine isolates tested. Recently cloned immunodominant *Neospora* antigens were used to develop a recombinant antigen ELISA which proved to have increased sensitivity for the detection of infection in cattle when compared to the standard tachyzoite lysate ELISA. A PCR amplification and probe system developed for the detection of *Neospora* in bovine tissues and fluids was used to identify parasites in chronically infected cattle, thus providing new insights into the potential mechanisms of vertical transmission of *Neospora*.

Amino acid supplementation of lambs infected with *Teladorsagia circumcincta*

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Protein supplementation has been shown to influence the resilience and resistance of young sheep to gastrointestinal nematode infection.

Forty eight 5.5 month-old worm-free lambs were allocated to six equal groups. Groups 1, 3 and 5 were offered *ad-libitum* a ruminant ration containing moderate protein (120 g CP Kg⁻¹ FM), high protein (180 g CP Kg⁻¹ FM) or the moderate protein supplemented with protected methionine (2g Kg⁻¹ FM) respectively and infected daily with 2000 *T. circumcincta* L3 for 8 weeks. Groups 2, 4 and 6 were uninfected controls pair-fed to groups 1, 3 and 5 respectively. All the lambs were treated with anthelmintic at week 8, challenged at week 9 with 50,000 *T. circumcincta* L3 and killed 10 days later.

The rate of liveweight gain over the first 8 weeks was higher in the pair-fed controls than in the respective infected groups. There were no significant differences in faecal egg output between groups 1, 3 and 5 although the mean egg count tended to be higher in the methionine supplemented group. The mean total worm burdens and their stage of development in groups 1, 3 and 5 were lower/reduced compared to the respective challenge controls but there were no dietary interactions. Dietary treatment did not significantly affect the establishment of the challenge infection (58, 45 and 62% for groups 2, 4 and 6 respectively). The number of mast cells in the abomasal mucosa were higher in the previously infected lambs but the numbers were not significantly influenced by methionine supplementation. Protein or methionine supplementation possibly failed to influence the rate of development of resistance to infection as the protein requirements for growth would be lower in this age of lamb.

Immunopathogenesis of murine infections with human *Babesia* isolates

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Two species of hemoprotozoan babesial parasites, *Babesia microti* and WA1, are known to infect humans in the U.S.A. These parasites are morphologically similar but molecularly and biologically distinct. Infection of mice with either parasite results in the development of parasitemia (2-10%). However, the WA1 isolate causes fatal disease in rodents, whereas little or no pathology is associated with *B. microti* infections. The difference in pathogenesis of these *Babesia* species provides a system to identify T cell subsets and associated cytokines which mediate pathology or resolution of infection. Our study tested the hypothesis that an overproduction of TNF α contributes to the death of WA1-infected mice. The kinetics of TNF α , IFN γ , IL-10 and IL-4 production in T cells were measured during WA1 and *B. microti* infections in C3H/HeN mice using flow cytometry. WA1-infected mice showed increased production of TNF α by $\gamma\delta$ T cells throughout the infection, while *B. microti*-infected mice showed increased production of IL-4 by $\gamma\delta$ T cells. In both groups IFN γ was produced by CD8⁺ T cells. These results indicate that TNF α may play a role in the pathology of a WA1 infection, while IL-4 is associated with resolution. IFN γ may be important for parasite killing. In another experiment, mice that were immunized with *B. microti* and challenged with a lethal dose of WA1 resolved the WA1 infection. The flow cytometry results showed that the cytokine profile of these mice was similar to the *B. microti*-infected mice. These results support the idea that protection is associated with the absence of TNF α and the presence of IL-4. Survival of lethal WA1 inoculations in TNF α R1 knockout mice have further demonstrated that the absence of TNF α is beneficial to the mice.

The use of an eprinomectin pour on formulation against endoparasite infections in cattle

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The new generation macrocyclic lactone parasiticide, eprinomectin, was selected for development as a topically applied endectocide for cattle based on potency, safety and a favorable metabolic disposition. The latter characteristics permit zero milk withholding and in some countries, zero meat withdrawal. Thirty seven studies were conducted to examine the efficacy of the formulation against adult and fourth-stage larvae (L4) of all common cattle nematodes including *Haemonchus placei*, *Ostertagia ostertagi*, *Cooperia* spp, *Trichostrongylus axei*, *Nematodirus helvetianus*, *Oesophagostomum radiatum*, *Dictyocaulus viviparus* and a number of less common nematode species. For adult parasites, studies were conducted with induced and natural infections. Where natural infections were used, animals were generally housed for a period before treatment under conditions precluding further nematode infection which allowed development of most non-hypobiotic larvae so that the nematode stage present at treatment was more correctly defined. For L4 of most species, induced infections were used to ensure that the required developmental stage was present at treatment. Cattle were randomly assigned to treatment groups from within a ranking on fecal egg counts or body weights. All studies included an unmedicated control group and a group treated with eprinomectin applied topically at 500mcg/kg body weight. In most studies, the animals were housed in individual pens or stanchions. Necropsies were conducted for nematode recovery 2 to 4 weeks after treatment. Overall efficacy was 97 % ($p=0.001$) for *Trichuris* spp and >99 % ($p=0.001$) for all other species. The results of this extensive program confirm the excellent efficacy of the eprinomectin topical formulation against various strains and stages of endoparasites in cattle.



Trypanotolerance and trypanotolerant livestock

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Trypanotolerance, the ability of livestock to survive and to remain productive under a major trypanosomiasis risk without the aid of trypanocidal drugs, is an attribute of breeds of cattle, sheep and goat in west and central Africa. Trypanotolerance in cattle is well documented, particularly in NDama cattle, the most numerous trypanotolerant cattle and in the west African Shorthorn. The exploitation of these breeds is practised as a major if not the only sustainable option for livestock production in the wettest parts of west and central Africa. They were successfully introduced in areas of west and central Africa where cattle breeding had not been possible before because not only of high trypanosomiasis risk but also due to high risk of dermatophilosis. Trypanotolerance of sheep and goat has been less well documented and needs further investigation particularly its correlation with helminth resistance.

Major constraints to putting genetic resistance into practical approaches for selection for trypanotolerance, within breed or in cross-breeding programmes, have been the absence of reliable practical indicators of trypanotolerance. Trypanotolerance has been associated with two major components, the ability to control the parasite and the ability to control the development of anaemia. Genetic parameters have only been estimated, although not yet precisely, for anaemia control (packed red cell volume). Practical indicators of the parasite control capability have still to be identified; they rely on the availability of more precise diagnostic techniques that could be carried out at the farm level. Alternative predictors, including molecular genetic markers, are being investigated.

Genetic correlation between these indicators, the production traits and resistance to other economically important parasites/diseases have still to be determined. As dermatophilosis is often present in the wettest trypanosomiasis affected regions, research on practical indicators of resistance to that disease is required to establish relevant integrated strategies based on disease resistant livestock. Economic assessment of these and alternative strategies will have to contribute to decisions on most appropriate options for given production systems.

063

Detection and duration of colostral antibodies to equine piroplasmiasis and the incidence of new infection, in foals in South Africa

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Equine piroplasmiasis, caused by *Babesia equi* and *Babesia caballi*, is widespread in South Africa. *B. equi* is also an important cause of abortions and stillbirths due to transplacental infection of the foetus. The prevalence and duration of maternally derived antibodies to *B. equi* and *B. caballi* were studied in 3 groups of foals (n=72) using the indirect immunofluorescent antibody (IFA) test. Two groups of foals, from different equine piroplasmiasis endemic areas (prevalence of *B. equi* = 74-98.3 % and *B. caballi* = 1.7-22% in yearling foals), and a third group - born and reared under tick free conditions, were used in this study.

Pre-colostrum samples from most foals (9/15) tested negative to *B. equi* and *B. caballi*, while being positive after colostrum intake, comparable to that of the dams. The duration of colostral antibodies in foals varied from 11-210 (mean = 79 ± 45) and 11-160 (mean = 64 ± 42) days for *B. equi* and *B. caballi*, respectively.

Natural infection (of both *B. equi* and *B. caballi*) occurred in these foals between 41 and 328 days of age, as indicated by positive seroconversion. No overt sign of clinical disease was observed in any of the foals, indicating a period of natural immunity to equine piroplasmiasis due to maternally derived antibodies and possibly other nonspecific factors.

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Protective immunity against *Fasciola hepatica* infection in cattle Th1 or Th2?

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Infection of farmed ruminants with the liver flukes, *Fasciola hepatica* in temperate regions, and *Fasciola gigantica* in the tropics, causes widespread disease and also sub-clinical economic losses. While acute disease, is common in sheep, cattle generally suffer only sub-acute or chronic disease. The bovine immune response to infection does not protect against a further, low-level, challenge infection. The flukes secrete enzymes which help protect against antibody-mediated killing. Certain of these enzymes (Cathepsin L1 and L2) have been characterised and purified. Purified CL1 and CL2, in oil adjuvant, reproducibly confer a significant degree of protection to cattle against experimental challenge infection. The immune response of animals vaccinated according to this protocol is qualitatively, as well as quantitatively, different from that of unvaccinated animals. Lymphocyte responsiveness to *F. hepatica* antigens, for example, correlates positively with fluke burden in chronically-infected cattle, but negatively in cattle immunised with CL1 and CL2. We propose that the response induced by infection is a classical Th2-type response, whereas the response to our vaccination protocol is more akin to a Th1 response. Accordingly, we would expect cytokine production pattern by responding lymphocytes to be different if our hypothesis is correct. γ -INF production by peripheral blood lymphocytes restimulated by *F. hepatica* antigen *in vitro* occurs only up to week four post-infection. However, this same assay performed using blood from cattle vaccinated with CL1 and CL2 showed a γ -INF response eleven weeks after initial immunisation. Preliminary results of RT-PCR analysis of IL-4 and γ -INF mRNA in hepatic lymph nodes of cattle during the first ten weeks of an infection indicate that IL-4 production continues as γ -INF production declines. Our studies to date have shown that it is possible to provide significant protection to cattle against *F. hepatica* infection not by reproducing the response induced by infection, but by a very different type of response. Thus, the lack of acquired immunity following infection is not an obstacle, and the prospects for vaccination in sheep, as well as in cattle, are therefore good.

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Use of a live attenuated vaccine for control of bovine babesiosis in Australia

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A highly effective, live vaccine is used in Australia for the control of bovine babesiosis. Production procedures are well documented and, since 1966, 30 million doses have been provided. The nature of the product and changing requirements since 1990 for quality control and product registration have presented challenges unique in the field of immunobiologicals. Through research and adoption of strict production standards, the challenges are being met.

Compliance with the Code of Good Manufacturing Practice is being achieved through a total review of production procedures and the upgrading of facilities at a cost of US\$ 3 million.

The potential demand in endemic areas is 1.2 million doses pa. rendering the product commercially unattractive. As a result, the Queensland Government is continuing to support production based on a user/provider model with emphasis on cost-recovery.

In general, the vaccine is highly effective with one vaccination providing lasting protection. Vaccine failures have been reported in certain breeds of cattle and form the basis of a major research project.

In the absence of alternative vaccines of acceptable potency and cost, the live vaccine continues to play an important role in support of Australian livestock industries with benefits from its use amounting to US\$ 20 million pa. Recent developments have confirmed that it can be produced cost-effectively and to cGMP standards while meeting the needs of consumers.

Faecal egg examination as an indicator of the worm burden and morbidity in early *Schistosoma mattheei* infections in cattle

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The lesions of schistosomiasis are not caused by the adult worms which are lodged in the veins of the host, but largely by their eggs being trapped in tissues. A major factor restricting epidemiological studies on schistosomiasis is that it is not possible to count those worms or tissue eggs *ante-mortem* in infected subjects. Predictions of internal burdens made from faecal egg counts would be of great use in our understanding of schistosome infections.

In endemic areas, an immune-related decline in faecal egg production is generally observed in cattle older than 18 months. Therefore, the present study examined *Schistosoma mattheei* adult female worm burdens (WP), total tissue egg counts (TEC) and faecal egg counts (EPG) from 30 Friesian calves aged less than 18 months at slaughter. The calves had been naturally exposed to infection over periods of time ranging between 2 to 12 months. A significant positive relationship between log transformed WP and EPG was found in all 3 groups. Using the overall geometric mean burden of 90 WP, the overall EPG to WP ratio was calculated as 0.32:1. A significant positive relationship was also observed between the log transformed TEC and EPG, with an overall EPG to TEC ratio of 0.00063:1 calculated from the overall geometric mean of 445000 tissue eggs. The overall relationship between WP and TEC was more complex, and followed a sigmoidal pattern. At low adult female worm numbers there was an exponential increase in TEC. This rate of increase began to level off around 200 WP, resulting in a plateauing of TEC at higher burdens. The results clearly show that the intensity and morbidity of *S. mattheei* infection in cattle can be predicted from faecal egg counts during the first year of moderate natural infection. The decrease in EPG:WP ratio as worm burdens increase appears to be due to both a larger accumulation of eggs in tissues and a decrease in the fecundity of the female worms.

Taenia solium cysticercosis in pigs. Role of immunity induced by primo-infection.

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The immunity of rural pigs, related to *Taenia solium* has not been studied. It is not known, whether pigs, once infected, are immune to reinfection, and if so, for how long. A great deal of research has been carried out to confirm the presence of antibodies in experimentally and naturally infected pigs, but it has not been established whether these antibodies protect the animal against further infections. The present study was carried out with the objective to determine if pigs, infected at an early age, are immune to reinfection and how long the immunity lasts. Fourteen 2 to 3 months old piglets were orally infected each with 100,000 eggs from *T. solium* (T1). Three pigs were euthanized 2 months later to confirm the infectivity of T1. Five months later 5 of the pigs were reinfected with the same amount of eggs from a second tania (T2). Nine months after infection with T1, eggs from a 3rd tania (T3) were fed to the remaining 6 animals. Two months old control piglets were inoculated with eggs from T2 and T3. All pigs were kept in a technified, *T. solium* free piggery. The animals were bled monthly and ELISA as well as Western Blot were done. The animals were euthanized at different periods p.i., all metacestodes counted and classified in caseous, colloid and vesicular. The results show that 5 months after the first inoculation 4 of 5 animals did not become reinfected, the fifth showed slight infection compared with the controls, which were all heavily parasitized. Nine months after infection with T1 50% of the reinfected animals with T3 were heavily parasitized. In those animals that were protected by the first infection (T1) circulating IgG antibodies could not be demonstrated with ELISA nor with WB at the time of reinfection. In contrast, in those pigs that were less protected by the first infection, other circulating antibodies were present. These findings suggest that antibodies induced by the infection are not associated with protection. The results underscore the need to characterize the immune response associated with cysticercosis. It is also necessary to study the serology of free ranging animals living in endemic and hyperendemic areas.

Control of schistosomiasis in ruminants: potential of vaccination

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Schistosomiasis in ruminants are of great veterinary significance in some parts of the world and it is recognized that human infections with *Schistosoma japonicum* in the Far East can never be controlled without also controlling the parasite in ruminants. Obviously, the most effective way to control schistosomiasis in ruminants would be to prevent contact with potentially dangerous waters by, for example fencing, but such practice is often impossible to apply in the field. Although praziquantel is highly effective against all visceral schistosomes in ruminants, it is expensive and does not prevent reinfection. Therefore, chemotherapy is not really a suitable method of control in domestic stock in most parts of the world.

First attempts towards immunological control of schistosomiasis in ruminants focused on the use of irradiated vaccines. Attenuated live vaccines were shown to be protective both under laboratory and field conditions, but were not suitable for large-scale field application. The current research trend is to identify defined protective antigens that are easier to standardize and to deliver. Of the two vaccine candidates tested so far in ruminants (keyhole limpet haemocyanin (KLH) and glutathione S-transferase (GST)), the GST appears the most promising. Depending of the animal model used, immunization with GSTs induced either significant protection against challenge infection, or significant reductions in the fecundity of female worms and in egg hatchability. These results testify to the great potential of vaccination in controlling schistosomiasis in ruminants. However, recent trials showed that the level of protection induced by vaccination may largely depend on the pattern of challenge infection, suggesting that a vaccine could only be of value in certain epidemiological conditions. Therefore, epidemiological factors should be considered when evaluating the potential of vaccine candidates against human or cattle schistosomiasis.

Moxidectin pour-on : study designs and persistent efficacy

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Persistent efficacy of anthelmintic compounds against pathogenic nematodes is an important feature in the control of nematodes and in the determination of strategic programs

The persistent efficacy of moxidectin pour-on has been tested under different study designs. In the first design, groups of animals were treated at different times before a single induced challenge was administered and animals were necropsied for worm counts 3 weeks after the challenge. In the second design the animals were treated and slaughtered as before but animals were challenged naturally by grazing 3 consecutive days on a contaminated pasture. Finally, a 3rd design was used where groups of animals were treated followed 3 to 4 weeks later by daily induced infections during another 17 to 22 days. The animals were slaughtered for worm counts 5 days after the last challenge. The early slaughter allowed efficacy to be determined against various worm stages and so to measure efficacies over different periods with only one treated and one control group.

The protection against *Ostertagia ostertagi* and *Dictyocaulus viviparus* in the different study designs are shown in the table below :

Persistent efficacy (%) on day post treatment

Nr. Days	<i>Ostertagia ostertagi</i>			<i>Dictyocaulus viviparus</i>		
	28	(28-)*35	(35-)*42	(21-)*28	(28-)*35	(35-)*42
Design 1	>99	>99	97.5	>99	>99	95
Design 2	100	>99	N.T.	100	98	N.T.
Design 3	N.T.	>99	>99	100	100	100

*for the third study design, periods have to be considered N.T. not tested

In the 3 study designs the results were similar showing that moxidectin pour-on provided $\geq 95\%$ protection against both *Ostertagia ostertagi* and *Dictyocaulus viviparus* for at least 42 days this may allow to extend the period between two treatments in strategic programs.



Early determination of trypanotolerance level of cattle using a standardised monovariant *Trypanosoma brucei* infection

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Trypanotolerance level of Borgou, Lagune, White Fulani Zebu and Holstein cattle was evaluated following subcutaneous inoculation of variant AnTat 1.1E of *Trypanosoma brucei*. Parasitaemia in Holstein and Zebu was persistent whereas in Borgou and Lagune it was of a short duration. Infected animals showed a drop in PCV which was most pronounced in Holstein cattle. Trypanolytic antibodies were detected in sera of all infected animals. In the early period of infection, complement titres dropped down followed by total or partial recovery or no recovery at all. Based on parasitaemia and clinical signs, Borgou and Lagune breeds are classified as tolerant, Zebu as subtolerant and Holstein as sensitive to trypanosome infection. However, there was a large variability within the breeds. Using the criteria of parasitaemia as superior to 1 trypanosome per microscopic field (400x) on day 15 and at least 10% drop in PCV on day 15 (PCV d15 - PCV d0) all sensitive individuals may be identified and treated in early stage of infection on day 15. Using the additional criteria of at least 10% drop in the complement titre between day 15 and day 20, the individuals may be classified into either tolerant or sub-tolerant group. Thus using the appropriate cut off points for drop in PCV on day 15 and drop in complement on day 20, the sensitivity and specificity of the test for classification of animals into sensitive, tolerant or sub-tolerant, category become 100%. As there was no correlation between the variations in PCV and complement, it is assumed that the two aspects of infection are under different genetic control. It can be concluded that the standardised infection by *T.b.brucei* AnTat 1.1 provides a reliable tool to measure the trypanotolerance level of cattle within 20 days.

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Serum antibody a poor indicator of level of resistance of *Haemonchus contortus* infection in lambs

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The identification of resistant or responsive sheep to haemonchosis is currently based on measuring faecal egg counts (FEC) following a natural or an artificial challenge. Searches are underway for more efficient methods. One such method could be the analysis of specific antibodies from blood samples. It has been suggested that antibodies play an important role in genetically determined resistance of sheep to *Haemonchus contortus*. Here we present the results of two infection trials in different tropical genotypes of lambs. In a first trial, lambs received a trickle infection with 6000 L3 of *H. contortus*. They were monitored for 10 weeks after which they were slaughtered for worm counts. In a second trial a similar primary infection was terminated 50 days post infection by anthelmintic treatment and was followed by a challenge infection with 12,000 L3 of *H. contortus*. The course of the infections was monitored weekly by using parasitological, biochemical and serological parameters. Excretory/secretory products of adult *H. contortus* were used as the antigen in an ELISA. High establishment rates were observed in all genotypes. Strong relationships between worm counts, faecal egg counts and PCV were demonstrated. Although a significant increase in mean serum IgG levels was measured following infection in all genotypes in both trials, no relationship between establishment of infection and antibody level could be shown. It is concluded that measuring specific serum IgG levels, following artificial infection with *H. contortus*, is of little value in identifying resistant individuals in random-bred sheep.

070

Seroprevalence of ovine and caprine oestrosis (*Oestrus ovis* Linné 1761) : survey on 2720 sera from eight african countries. Ph. Dorchie, Ph. Jacquet, C. Duranton, F. Prévot, J.P. Bergeaud
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The control of *Oestrus ovis* infection in sheep and goats should be included in integrated parasite control program in areas where it is a parasite of economic importance. Usually this infection is ignored or neglected because breeders and vets are used to seeing nasal discharge and hearing sneezing. This myiasis is often included in pasteurellosis syndrom or in pleuro-pneumonia symptoms.

An ELISA test has been developed and a survey has been done with random samples from sheep of Benin (21 sera), Burkina Faso (421), Djibouti (303), Ethiopia (95), Ivory Coast (62), Niger (92), Senegal (753), Togo (280). The percentage of infected animals were respectively : Benin 14%, Burkina Faso 86,3%, Djibouti 96,4%, Ethiopia 54,7%, Ivory Coast 68%, Niger 98,8%, Senegal 90,5% and Togo 88,6%.

From goats the numbers of samples were : Djibouti (176), Ethiopia (182), Senegal (335). The percentage of infected animals were respectively : Djibouti 99,4%, Ethiopia 89,6%, Senegal 94,4%.

In fields a lot of animals showed typical clinical signs. However there is no relationship between the amount of antibodies and severity of the infection. It is imperative to consider that this parasite is a severe threat for small ruminant breeding in many countries mainly where the climate is hot and dry.

Furthermore during the survey many breeders suffered from human extern oculo-myiasis due to *Oestrus ovis* larvæ laid on their eyes. These results clearly indicate that this infection may be far more important than it was previously thought for sheep and goats as for human beings.

072

Serum pepsinogen levels and measuring the exposure to gastrointestinal nematodes in first-grazing season calves

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Quantifying the exposure of first-grazing season (FGS) calves to gastrointestinal nematode infections would be of great use in evaluating the efficacy of a particular control programme. However, there are only a few parameters available for evaluating what host-parasite contact has occurred over a grazing season. One possible parameter is serum pepsinogen determination at housing. Unfortunately the methods used differ markedly between laboratories and the observed values for a given pepsinogen level are also very different. Moreover, the actual methods are labourious and expensive, making them impractical for routine analysis. Here we present a simplified micro method that is based on the method of Berghen *et al.* 1987 (Am. J. Vet. Res., 48: 664) but has the great advantage of only using small volumes of samples and reagents. Using this technique the serum pepsinogen levels of 41 groups of FGS calves were determined at housing. The grazing history and chemoprophylactic systems used were known for all groups, and levels of infection had been monitored throughout the grazing season for all calves. There were very clear differences in the mean pepsinogen levels at housing between chemoprophylactic treated groups (between 0.5 - 2.5 U tyrosine), non-treated groups in which no clinical signs of parasitic gastro-enteritis (PGE) had occurred (between 2.0 - 4.1 U tyrosine), and non-treated clinical PGE groups (between 3.4 - 6.3 U tyrosine). In the chemoprophylactic treated groups there was a significant negative relationship between mean serum pepsinogen levels at housing and the duration of chemoprophylaxis.

The epidemiology and socio-economic effects of *Echinococcus granulosus* in mid-Wales and northern Jordan.

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Hydatid disease is a global zoonosis caused by the canine tapeworm *Echinococcus granulosus*. A comparative epidemiological and economic study of this disease was carried out in the endemic areas of mid-Wales and northern Jordan - two areas selected as representative of developed and developing economies respectively.

A random survey of farm-owners and labourers was conducted in order to obtain data on age, sex, status farming practices, dog ownership etc. Hydatid cases identified were cross-matched for sex and age and risk factors, both cultural and socio-economic tested for significant association.

Hospital case records, where available, were obtained for hydatid patients and used to approximate the financial cost of treatment. The extent to which hydatid disease affects the quality of life of patients was assessed using the novel approach of Brazier's Short Form 36 Health Status Questionnaire (SF-36).

Preliminary analyses of risk factors were found to be inconsistent with work previously done, and strongly suggests the presence of confounders.

Cost analysis of treatment for hydatid disease illustrated much higher overall costs in Wales compared to Jordan. This, we believe, is directly linked to the payment of higher wages to specialised personnel in developed countries and the willingness-to-pay by the patient.

Quality of life (QoL) surveys were used to examine data relating to the patient's own opinion of his/her's current health status. Analyses showed large differences between groups and within groups. One of the most noteworthy findings was the low quality of life score recorded among females in Jordan. Hydatid positive females scored lowest overall and hydatid negative females reported a even lower QoL score than Hydatid positive males.

With further studies, it is hoped to identify priorities for the investment in hydatid control in both developed and developing countries compared to the prevention of other diseases.

The importance of tick infestations to cattle owners in urban, small-scale farming communities

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The purpose of the study was to determine the nature and extent of tick related problems to urban, small-scale farming communities. The study was conducted in the cities Botschabelo and Thaba Nchu where site were selected to include cattle grazing within urban areas, on urban outskirts and those kept in more traditional village management systems. Between 55 % and 60 % of the live stock kept in these areas are cattle. The study included tick collections from 50 animals over a 12 month period, serological tests on blood samples from selected animals (n = 151) and questionnaires completed by 200 farmers. Ten species of ticks occurred on the cattle and *Boophilus decoloratus* (87.26 %), *Rhipicephalus evertsi evertsi* (8.86 %) and *Hyalomma marginatum rufipes* (2.42 %) were the most abundant. Seasonal patterns of abundance were evident for most of the species, *B. decoloratus* being the most abundant during the cooler months of fall and early winter, whereas most of the other species were most abundant during the summer months serological tests showed that the animals tested (n = 151) had an average seroprevalence of 62.42 % to *Babesia bigemina*, 19.47 % to *B. bovis* and 98.6 % to *Anaplasma marginale*. The questionnaires showed that 88 % of the cattle owners were aware of the presence of ticks on their animals and that 73 % practised some kind of tick control : 45 % used registered acaricides, 19 % used old crank case oil and 5 % Jeyes fluid. The findings of the study make it possible to propose a program of integrated control.

Predation of livestock ticks by chickens as a tick control method in a resource-poor urban environment

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The possible use of chickens as predators of livestock ticks was studied. Chickens were allowed to scavenge for three hours among tick infested cattle in a typical township backyard during the milking period. *Boophilus decoloratus*, *Hyalomma marginatum rufipes*, *Rhipicephalus evertsi evertsi* and *Otobius megnini* were recovered from crops and gizzards at necropsy. The numbers of ticks ingested ranged from 0 to 128, with an average of 28.81 (8.42 per chicken). This study has confirmed that chickens are natural predators of livestock ticks and that chickens can be used as part of an integrated tick control plan in urban cattle management systems in resource poor communities in South Africa.

Efficacy of a new compound, imidacloprid, against fleas in a field trial in dogs and cats.

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This study was conducted to evaluate the effectiveness of topically applied imidacloprid and the combination of orally administered lufenuron and a pyrethrin flea spray to control flea infestations. Dogs and cats in 34 flea-infested homes in Tampa FL (USA) were selected between May 20 and June 20, 1996. Pets in group 1 were treated topically with 9.1% imidacloprid (8.9 - 25.1mg/kg) spot application on day 0 then once every 28 - 30 days for 90 days. Pets placed into group 2 were given oral lufenuron (dogs: 10 - 22.5mg/kg; cats 27.5 - 59mg/kg) once every 28 - 30 days for 90 days and were treated topically with a pyrethrin flea spray once every 1 to 2 weeks. Flea populations were assessed in the environment using an intermittent light trap, pet flea burdens were assessed using visual area counts. On day 0, the flea traps collected an average of 43.5 (± 75.3) and 59.9 (± 80.2) fleas from the 19 homes in group 1 and the 15 homes in group 2, respectively. Pets in group 1, had an average of 16.6 (± 13.7) fleas observed in area counts. Pets in group 2, had an average of 26.4 (± 30.6) fleas observed in area counts. Three households randomly allocated to group 2 were switched during the study to Group 1 due to client dissatisfaction. A single application of imidacloprid was 96% and 93.5% effective in reducing flea populations on pets at 7 and 28 days, respectively. Following 3 applications (84 - 90 days) flea burdens on pets and in the environment were reduced by 98.8% and 99.9%, respectively. The combination of lufenuron and weekly pyrethrin spray were 48.9% and 91.1%, effective in reducing flea populations on pets at 7 and 28 days, respectively. This combination reduced flea burdens on pets and in the environment by 99.2% and 99.7%, respectively, by days 84-90.

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Toxoplasma gondii, *Neospora caninum*, and certain species of *Sarcocystis* are apicomplexans known to cause abortion and neonatal mortality in livestock. The structure of the parasite, serological examination, detection of parasite DNA, immunohistochemical tests, or sometimes a combination of these, are needed for a definitive diagnosis. *T. gondii* and *N. caninum* tachyzoites are similar but their tissue cysts can be distinguished; *N. caninum* tissue cyst walls are 1-4 µm thick whereas the cyst wall of *T. gondii* is <1.0 µm thick. Serological examination of the dam is not diagnostic for abortion due to toxoplasmosis, neosporosis or sarcocystosis because of high prevalence of these parasites in the general population. Finding antibody to *T. gondii* or *N. caninum* in fetal fluid is diagnostic whereas a negative result is not; thus, not finding antibody does not rule out infection. Immunohistochemical tests using specific sera is the most practical method at the present time to detect parasites in fetal tissues. Specific primers are available to distinguish *N. caninum* from *T. gondii* by polymerase chain reaction (PCR). *Sarcocystis* organisms in fetal tissues can be distinguished by their structure, even by the light microscope. At present there are no reliable serological tests for the diagnosis of *Sarcocystis*-associated abortion.

The forming of strongylids' community and cumulation in the host effect of parasites of equids

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The study of patterns of forming of strongylids' community in horses opens the possibility to understand the nature of stability of host-parasite systems and mechanisms of their functioning.

Evaluating the strongylids' community from the position of evolutionary parasitology gives basis to state that connections between strongylids and their hosts were formed and intensified as portion of grasses in the nutrition of equids was raised. These connections completely were formed at the time obligatory grassing Equidae appeared.

The sequence of horses invasion by cyathostomes and strongylids reflects with high probability evolution steps of forming of their community.

The comparison of phylogeny of equids and strongylids gives reason to say that taxonomic and ecological structure of the community reflects with some approximation the history of relationship of hosts and their parasites on the last step of their coevolution. At the time Equidae were occupying the steppe ecosystems, ecological close, concurrent species of hosts were connected as symbiotic systems. That was reason for host cumulating of parasites.

This process was repeated: during the supplanting of *Hipparion* by unidactyl horses most parasite systems became extinct. However at the phylogenetic branch of contemporary horses the cumulation is reflected in high degree: there are more than 65 species and 21 genus that have passed a long evolution way from different taxons of equids.

The gathering of close relative species of strongylids in large gut of horses was reason of strengthening of significance of these parasites for regulation of host population.

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The wild boars have always been the source of human trichinellosis in the Slovak Republic. Therefore wild boars and red foxes were studied for their role in the maintenance of the sylvatic cycle of trichinellosis. The muscles were examined by a digestion method for the presence of *Trichinella* larvae. Serum from wild boars and thoracic transudate from red foxes were examined by ELISA for the presence of anti-*Trichinella* antibodies. *Trichinella* spp. larvae were most frequently detected in *Ursus arctos* (20 %), *Canis lupus* (7.1 %) and *Vulpes vulpes* (3.2 %). Other wild and domestic carnivores examined were found negative. Of wild carnivores, fox is the only animal occurring over the entire territory. Therefore 1349 samples were examined from foxes shot in 1994-1996 to check the efficacy of antirabic vaccination. Anti-*Trichinella* antibodies were detected in 25.9 % of the foxes examined. In the years 1954-1956 no trichinellosis was detected in wild boars. Currently, 0.22 % of wild boars examined by a digestion method were diagnosed with trichinellosis on the average. The prevalence of trichinellosis fluctuated over those years from 0.06 % to 0.45 %. Trichinellosis was serologically diagnosed in as many as 15.9 % of wild boars. From the epidemiological point of view, trichinellosis in the Slovak Republic circulates among wild boars or red foxes in 32 districts of 37 and only 5 districts are trichinellosis-free. This wildlife, occurring over the entire territory, is the major maintainer of the natural sylvatic cycle of trichinellosis in the Slovak Republic.

The review of unresolved problems of taxonomy of Strongylidae of horses

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Almost 100 years ago the study of taxonomy of strongylids of horses was founded by A. Looss. At the present time more than 60 species are described, some large revisions were made. Nevertheless the number of questions is still need studying.

Different points of view on relations ancestor-descendant between Cyathostominae and Strongylinae are existed. This question is general for all Strongyloidea – what shape of buccal capsule – round or cylindrical – is the plesiomorphic character?

Though the opinion about Cyathostominae of horses as monophyletic taxon is generally accepted, phylogenetic relations between Cyathostominae and Strongylinae of horses and such helminths of other hosts ought to analyze once more. It is very tempting to pick the group of parasites of one host out to separate taxon. Strongylinae genera *Triodontophorus*, *Craterostomum* and possibly *Bidentostomum* having round buccal capsule, the same life cycle and morphology of IV stage larvae are very similar to Cyathostominae. So the possibility of addition this group to the latter was discovered by A. Railliet (1923) many years ago.

Cyathostominae is a good illustration for unification and dividing approaches in taxonomy. According to different authors here is from 10 to 14 genera accounted. The separation of genera *Coronocyclus* from *Cyathostomum* is acknowledged yet. The authors concerned that genera *Cylicotetrapodon*, *Skrijabinodontus*, *Tridentoinfundibulum* and also *Parapoterostomum* should be concerned like separate genera. Unification the former these genera with *Cylicostephanus* and the latter with *Cylicodontophorus* is only the contribution to tradition.

Last years some new species of strongylids of horses were described. Following our experience Asia and Africa have perspectives in this respect. At the same time genus *Cylindropharynx* and species were described by Ricci (1939) from zebras are needed the redescription.

The authors suppose that studying of morphology of larvae stages of strongylids of horses and other hosts and analysis of their DNA with following working up of results by methods of characteristics could be promoted in solution of these problems.

The efficacy of topically applied eprinomectin for treatment of *Chorioptes bovis* infestations

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Mites of the genus *Chorioptes* inhabit the surface layers of the skin causing pruritus and in severe cases, the formation of thick, heavy scabs at the site of infestation which present a barrier to effective treatment. Nine controlled studies were conducted to investigate the efficacy of a topical formulation of the new generation macrocyclic lactone, eprinomectin, against *Chorioptes bovis* infestations. Dairy cows carrying natural *C. bovis* infestations were randomly allocated to treatment groups from a ranking based on mite counts or body weight. In each study, one group of 6 or 10 cows was treated with eprinomectin applied at 500mcg/kg (1mL/10kg) topically along the backline from withers to tailhead and a second similar group acted as unmedicated controls. Mites were counted in skin scrapings taken before treatment and at weekly intervals for 8 weeks after treatment. Scrapings were made from one to 6 sites at the edge of active or previously active lesions. In every study, the eprinomectin-treated group had significantly ($p < 0.05$) fewer mites than the control group at each scraping from Week 2 through the end of the trial at Week 8. There was $> 98\%$ reduction in mites at Week 2 and 100% reduction at Week 8. *C. bovis* infestations were also present in a study conducted during the field efficacy section of the development program. Mature dairy cows belonging to a commercial herd were allocated to replicates of 5 animals from which one animal was randomly selected as a control and the other 4 were each treated with eprinomectin administered topically at approximately 1mL/10kg. Body weights were estimated and the dose calculated and administered by the herd's owner. Mite counts were conducted pre-treatment and for 8 weeks thereafter using the same procedures as the controlled efficacy studies. No mites were found on any eprinomectin-treated animal at any post-treatment count. The results confirm the exceptional efficacy of this new macrocyclic lactone against one of the most refractory ectoparasites of cattle.

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Comparative efficacy study among moxidectin, ivermectin and doramectin against ivermectin resistant *Haemonchus contortus* strain in artificially infected sheep in Brazil

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Lambs 7-8 months old were housed and treated with anthelmintics to eliminate the original nematode infection. Eleven days, after the last anthelmintic treatment, a group of 40 lambs was infected with 5,000L3 of an ivermectin susceptible strain of *Haemonchus contortus* (S-IVM) and another group of 41 animals were infected with 5,000L3 of a known ivermectin resistant strain of *H. contortus* (R-IVM). At day 28 post-infection animals in Groups R and S were ranked by their egg counts, the animal with the lowest egg count within the R group was discarded; all animals were then allocated at random to one of four treatment sub groups (10 animals each) and medicated as follows: doramectin 0.2mg/kg, ivermectin 0.2mg/kg, moxidectin 0.2mg/kg and untreated controls.

Results from necropsies 14 days after treatment revealed that all three drugs used were highly efficient in controlling the susceptible strain. There were no differences ($P > 0.05$) between the efficacies of doramectin (99.2 %) and ivermectin (98.29 %) whereas moxidectin was 100% ($P < 0.05$). On the resistant strain, ivermectin had no effect while doramectin, used for first time, was only 18.94 %. Moxidectin, also used for the first time on this strain, was highly effective (99.93 %).

The above results demonstrate that *H. contortus* that are resistant to ivermectin are also resistant to doramectin. Moxidectin is highly effective in controlling these parasites.

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Efficacy of topically applied eprinomectin against the biting louse *Damalinia (Bovicola) bovis*

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Seven studies were conducted which examined the efficacy of the new generation macrocyclic lactone endectocide, eprinomectin, against the cattle biting louse, *Damalinia bovis*. In all studies naturally infested cattle were randomly allocated from within a ranking based on louse counts or bodyweight to treatment groups of 6 to 8 animals. Each study included an unmedicated control group and a group treated with eprinomectin applied topically at 500mcg/kg body weight (1mL/10kg) along the backline from withers to tailhead. Throughout the trials the cattle were housed in individual pens or stanchions which prevented contact between adjacent animals. *D. bovis* were counted in pre-determined sites before treatment and at approximately weekly intervals for 8 weeks after treatment. At the first post-treatment count on Day 7 there was 100% reduction in *D. bovis* in five studies and $> 94\%$ reduction in the remaining two studies. No *D. bovis* were found on any of the treated animals from Day 21 onwards through trial termination on Day 55/56. In five studies, the eprinomectin-treated group had significantly fewer ($p < 0.05$) *D. bovis* compared with controls at all post-treatment counts. In the other two studies, *D. bovis* counts were significantly lower in the eprinomectin-treated group ($p < 0.05$) on all but one or two occasions when there were low counts in the control animals. The results demonstrate that eprinomectin applied topically to cattle at 500mcg/kg provides excellent efficacy against biting lice.

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Pyriproxyfen effect on immature *Rhipicephalus sanguineus* ticks by on-animal application

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Pyriproxyfen (PPF) has been shown by the authors to alter hatchability and larval development from eggs laid by ticks fed on treated animals. In this communication its effect on immature stages of *Rhipicephalus sanguineus* (Latreille) is reported.

40 rabbits had their back shaved and were split into 8 groups of 5 each. PPF was sprayed at the following concentrations on the shaved area: 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 mcg/cm² of skin. A negative control group (no treatment) and a positive one (1mg of PPF/cm²) were included. Twenty four hours later, 50 larvae and 50 nymphs were put inside a closed dish stuck onto the skin of each rabbit. After natural detachment, the ticks were maintained in controlled conditions (30°C, 90% RH, darkness) for molting. Feeding and molting larvae and nymphs were counted.

Feeding behavior was not altered but molting was affected by PPF at 0.4 mcg/cm² and above, the effect increasing with dosage, supporting the implementation of PPF for integrated tick control.

group	larvae that (n)		nymph that (n)	
	fed	molted	fed	molted
control -	47.6 ± 3.80	47.0 ± 3.50	49.6 ± 0.50	48.2 ± 1.30
control +	46.1 ± 1.58	0	44.8 ± 0.44	0
0.1 mcg	49.6 ± 0.90	48.0 ± 1.60	48.8 ± 1.30	47.2 ± 1.60
0.2 mcg	49.4 ± 0.50	47.2 ± 1.80	49.6 ± 0.50	48.4 ± 1.10
0.4 mcg	49.8 ± 0.40	32.8 ± 2.60	48.0 ± 1.60	31.0 ± 1.60
0.8 mcg	49.0 ± 1.00	22.8 ± 2.90	48.4 ± 2.70	17.6 ± 2.10
1.6 mcg	47.6 ± 1.10	4.0 ± 2.70	46.2 ± 3.70	7.2 ± 7.40
3.2 mcg	46.2 ± 2.30	0	42.6 ± 2.40	0

Persistent efficacy of ivermectin* against gastrointestinal nematodes of cattle in Argentina.

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Thirty six parasite-free Friesian male calves, aged between 6-9 months old and with an average weight of 150 kg, were allocated to 6 groups of 6 animals each. On days -35, -28, -21, -14, -7 animals of groups 1, 2, 3, 4, and 5 respectively, were treated with Ivermectin 1.0% Injectable subcutaneously at 1.0 ml/50 kg body weight to give a dose of 0.2 mg Ivermectin.kg⁻¹ of body weight. The animals in Group 6 were considered as untreated controls.

On day 0, the 36 animals were experimentally infested with approximately 60000 freshly harvested infective larvae (L3) according to the following percentual composition: *Ostertagia ostertagi* 39 %, *Cooperia* spp. 17 %, *Trichostrongylus* spp. 25 %, *Haemonchus* spp. 12 %, *Oesophagostomum* spp. 7 %.

On days 26 and 27 replicate calves of Group 1, 2, 3, 4, 5 and 6 were necropsied and individual worm burden were determined.

Persistent efficacy (PE) against *Ostertagia ostertagi* adult forms and *Oesophagostomum* spp was 28 days post-treatment with a percentage efficacy of 99% ($p < 0.05$).

PE against *Haemonchus* spp., *T. axei* and *Cooperia* was 35 days post-treatment with a percentage efficacy of 99% ($p < 0.05$).

No adverse effects to the Ivermectin treatment, was observed in any group on the day of treatment, on days 1, 2, 3, 5, and 7 post-treatment and during all the trial.

Ivermectin* = Formulated as Bovifort by Biogenesis Sintyal.

Comparative productivity and efficacy of a two-dose program of doramectin or ivermectin for treatment of cattle in La Pampa, República Argentina

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Eighty Aberdeen Angus castrated males were randomly allocated to 2 groups of 40 animals each and grazed during a 240-day period in 2 homogeneous separate paddocks on Anquilovo Farm, Province of La Pampa, Argentina. Groups T1 and T2 were treated with doramectin and ivermectin (200 µg/kg of body weight, SC), respectively, on Days 0 and 60 post-treatment. Major objectives of the trial were to determine the effects of treatment on weight gain, pasture contamination, and infectivity at 130 and 240 days after treatment.

Throughout the course of the study, animals in the doramectin group had a mean accumulated EPG count of 173, whereas those in the ivermectin group had a mean accumulated EPG count of 235, as a measure of pasture contamination.

At 130 days post-treatment, the mean worm burden recovered from 4 principal animals in the doramectin-treatment group (3,675), was lower than that of principal animals in the ivermectin-treatment group (7,900). The mean worm burden at 240 days post-treatment was significantly ($P < 0.03$) lower for the doramectin-treatment group (7,725), compared with that of the ivermectin-treatment group (16,725). At the end of the trial (Day 240), cattle in Group T1 had a mean weight gain that was 14 kg higher ($P < 0.05$) than that of cattle in Group T2.

Main parasites recovered at necropsy of cattle from both groups included *Ostertagia* spp., *Trichostrongylus axei*, *Haemonchus* spp., *Cooperia oncophora*, and *Oesophagostomum radiatum*.

In the present study, doramectin showed excellent activity and efficacy in improving weight gain, decreasing fecal eggs counts, and decreasing worm burdens, in comparison to ivermectin.

The role of cats in the epidemiology of *Toxoplasma gondii* in Austria

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Strategies to prevent human toxoplasmosis are principally important for „seronegative“ pregnant women and individuals with continuous or temporary immune deficiencies. Since 1982 epidemiological studies were conducted to elucidate the roles of poorly prepared meat and infected cats as sources of human infection in Austria.

Due to modern pig management systems (improved standards of management and hygiene in piggeries and as a result of this much less contact with cats) the prevalence of *Toxoplasma* infections in pigs has decreased drastically during the last decade in Austria [1982: 13.7 % (n=2351), 1992: 0.9 % (n=2346)]. In Austrian wildboar populations infection rates were basically unchanged during this time [1983: 17.9 % (n=364); 1993: 19.3 % (n=289)]. Neither with direct nor with indirect methods could *Toxoplasma gondii* be diagnosed in 40 Austrian cattle. High infection rates of 66.4 % in Austrian sheep (n=4079) and 68.7 % in goats (n=687) were found in 1994 and 1995.

In order to elucidate the role of infected cats as a source of human infections 1368 cats were examined coproscopically by flotation method. Oocysts could be detected in only 2 % (n=1368) of cats. In order to obtain informations on the prevalence of antibodies against *T. gondii* in cats in Austria, 2002 blood samples of cats were collected in the years of 1996 and 1996 and tested by an indirect fluorescence antibody test (IFAT). The results of the study showed that in Austria 53 % of cats were infected with *T. gondii*. Prevalence of antibodies varied with age and type of cat.

Cats actively excreting oocysts make up a relatively small proportion (around 2 %) of the total tested cat population in Austria. On the other hand in seroepidemiological studies of cats prevalence rates of 53 % were found. This means that every second cat shed oocysts once in her lifetime. In addition it is noteworthy that an individual cat can shed hundreds of millions of oocysts, after infection. As oocysts are very resistant to environmental extremes, capable of surviving in the soil for some years, the danger of prolonged contamination and infection is evident. Austrian sheep are usually kept on small farms where cats abound and this also lead to a high prevalence of 66.4 % of *Toxoplasma* infection in sheep. Furthermore the popularity of eating mutton has increased throughout the last decade (1980: 190 819; 1993: 330 835). Although this supports the theory that sheep may be a very important factor for human infections, nevertheless cats may play an unexpected important role in the epidemiology of *T. gondii* in Austria.

Geographic Risk of *Fasciola* in animal, snail and human hosts in Kafr El Sheikh governorate, Egypt, with special reference to sources of human infection.

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To determine the source and species of *Fasciola* that infect humans and animals, and the species of snail that serves as intermediate hosts, six study sites were selected that were representative of thermal-moisture domains defined by AVHRR diurnal temperature difference maps of the Nile delta. Infections were found by fecal examination in 14.5 %, 26.6 %, 12.3 % and 6.0 % of buffaloes, sheep, cattle and humans, respectively. Incidence was higher in summer and autumn than in other seasons and higher in Sidi Salem and Motubus than in other study sites located in drier thermal-moisture domains. Examination of 100 *Fasciola* eggs from each host showed that egg size cannot be used as a reliable criterion for differentiating *F. hepatica* and *F. gigantica*. Upon dissection of 1972 *Lymnaea caillaudi*, 268 *L. alexandrina*, 502 *Bulinus* spp., 11316 *Biomphalaria alexandrina*, 1398 *Cleopatra* spp., 8520 *Physa* spp., 420 *Melania tuberculata*, 2132 *Vivipara* spp., 144 *Neritina nilotica* and 1570 *Planorbis philippi*, *Fasciola* pathenitae were detected in only *L. caillaudi*, the reported intermediate host of *Fasciola* in Egypt. Based on development in *L. caillaudi* and not *L. truncatula*, successive redial generations consistent with that described for *F. gigantica* were observed after infection with miracidia hatched from eggs of human hosts. Moreover, after infection with metacercariae from laboratory infected snails, early mature flukes recovered from laboratory infected rabbits had morphologic characteristics of *F. gigantica*.

Importance of parasitological research in the development of livestock in Egypt and Middle East

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The Egyptian livestock population has doubled in the last 30 years. Similar increases occur in its human population, which is expected to reach 62 million by the year 2000, and the country continues to face a shortage of livestock products especially milk and meat. Part of this shortage is related to production losses in the livestock production system, and these include losses due to parasitic and vector-borne diseases. Some of these losses are known to farmers and research establishment, but many subclinical diseases are not recognized.

The cornerstone of improvement is the development of human resources, and considerable improvement needs to be made in the training of veterinary practitioners and young researchers. This is in part related to poor infrastructure and funding for research, but also due to insufficient stimulation of young students (including high school students) in science.

With respect to parasitological research there are still major gaps in the understanding of the epidemiology of common parasites of Egypt and the Middle East in general. Since many of these parasites are the cause of zoonotic disease, their study requires collaboration between veterinary and public health researchers. Examples include the discovery and eradication of screwworm early this decade, the control of vector-borne diseases such as Rift Valley fever, West Nile fever and Crimean-Congo hemorrhagic fever, as well as schistosomiasis and liverfluke disease. Such collaboration requires planning and coordination of an interdisciplinary team or committee.

Finally it is important to stress the need to apply research results in the field and close the gap between research centres and research users; the latter includes the private sector. In this respect it is deemed important that users and beneficiaries participate in the planning of research, and ascertain the extension of research results and information to farmers and livestock owners.

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Biological and molecular characterization of *Toxocara canis* from the red fox (*Vulpes vulpes*)

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Helminth naive bitches were experimentally infected with *T. canis* isolated from red foxes (infection dose 20,000 embryonated eggs) on the day of conception. The pattern of infection was monitored coproscopically, clinically, hematologically and serologically until day 42 post partum. Additionally, genomic DNA of fox and dog isolates of *T. canis* were compared using RAPD (Random amplified polymorphic DNA) PCR. Furthermore sequences of ribosomal second internal spacer DNA (ITS2) was compared and homologies calculated. No significant differences could be found between infections with both isolates. RAPD-PCR patterns were identical with every primer used. DNA sequence homology of the ITS2 was 98%. The results show that both isolates are biologically and genetically identical. It may be assumed that regular transmission of *T. canis* between dogs and foxes prevents the development of genetically distinct fox and dog strains.

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Characterization of a recombinant *Ancylostoma caninum* antigen as an esophagus associated paramyosin homologue.

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Recombinant antigens of *A. caninum* were isolated from a λZAP II adult worm cDNA library by immunoscreening with polyclonal antisera against single organs (esophagus, cervical and cephalic glands, intestine). Clone 341 is a cDNA fragment with 518 bp size and codes for a 174 aminoacid polypeptide. The fusion protein reacted specifically in Western Blots with antisera against *A. caninum* esophagus. The DNA sequence of clone 341 shows homologies with paramyosins of *C. elegans* (85% homology with a 248bp segment, 80% with a segment of 183 bp, 81% with a 111 bp segment and 79% with a region of 196 bp of the unc-15 gene, 76% in a 130 and 78% in a 158 bp segment of clone cm2f8). Immunohistochemical characterization confirmed the location of the fusion protein in the esophagus. As paramyosin is known to be a protective antigen in *Schistosoma* clone 341 coding for a paramyosin homologue will be tested for its protective potential against *A. caninum* infections in dogs.

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Differential display reverse transcription - PCR (DDRT-PCR) shows specific cDNA populations in infective, hypobiotic and reactivated third stage larvae of *Ancylostoma caninum*

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Infective third stage larvae of *A. caninum*, hypobiotic L3 isolated after pepsinogen digestion of mice and dogs and reactivated lactogenic third stage larvae isolated from milk were collected from experimental infections. RNA was extracted using RNeasy™ Kit (Qiagen). After reverse transcription PCR was performed using anchor primers vs. random primer to amplify all cDNA populations according to a modified method described by Liang & Pardee (1994). The following gel electrophoresis of the amplicates of all anchor random primer combinations shows different patterns between infective, hypobiotic and reactivated larvae indicating different gene expression. Specific bands were eluted and sequenced to characterize cDNA fragments possibly involved in the regulation of hypobiosis.

Pharmacokinetics of ricobendazole after its subcutaneous administration to cattle at three different dose schemes

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Ricobendazole has recently been introduced in the veterinary market in Argentina. Based in pharmacokinetics and efficacy results, presented in separate communications in this Congress, we decided to carry on a complete pharmacokinetic study at three different dose levels, in comparison with the oral administration of albendazole.

Twenty four young parasitized castrated male Aberdeen Angus bovines weighting 150-250kg were used in the present experiment. The first group was constituted by six of the experimental animals that were injected subcutaneously with 4mg/kg ricobendazole (Sintytal R (r), Biogénesis-Sintyal, Argentina). The second group was formed by other six bovines receiving two 4mg/kg RBZ doses separated 48h. The six bovines of the third group received a S.C. 8mg/kg RBZ dose and the remaining six experimental animals received 8mg/kg oral albendazole. Blood samples from the injected animals were drawn at the following post-administration hours: 2, 6, 12, 24, 48, 72 and 96. RBZ and ABZ sulfone were extracted by liquid-liquid methodology and determined by HPLC with UV detection. The pharmacokinetic model that best fits the RBZ plasma profiles was discriminated by means of the MAICE test.

A one compartmental open model with first order absorption was discriminated as the best fitting the RBZ and ABZ sulfone plasma profiles according to the MAICE test for all the groups.

There are several interesting differences to establish between orally or parenterally administered benzimidazolic agents. When ABZ is administered orally its concentrations are undetectable and high levels of RBZ and sulfone are present, this is coincident with the majority of references in this subject. In the case of parenterally administered RBZ, we found an increase of persistence of serum concentrations that could be related with the lack of first pass effect and that permits to expect a pharmacokinetic advantage over oral pharmaceuticals that should coincide with elevated anthelmintic efficacy.

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Compared pharmacokinetics of ivermectin and moxidectin in goats

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The pharmacokinetic behaviour of antiparasitic drugs depends upon the route of administration. The formulation of the dosage form and the animal species. The antiparasitic spectrum and efficacy pattern for the different endectocide molecules are similar, however differences in physicochemical properties among them may account for differences in kinetic disposition and in potency or persistence of their antiparasitic activity. The aim of the present study was to determine and compare the disposition kinetics of ivermectin (IVM) and moxidectin (MXD) after oral and subcutaneous administration to goats at a dosage of 0.2mg/kg.

Twenty Murciano-Granadina goats were allocated in four groups of five animals receiving either parenteral or oral solutions of IVM and MXD. Plasma drug concentrations were measured by HPLC using fluorescence detection.

The absorption rate of MXD was not significantly different after oral and subcutaneous administration but the extent of absorption, reflected in the peak concentration (C_{max}) and the area under the concentration time curve (AUC), of the oral administration (15.52 ± 2.94 ng/ml and 33.98 ± 8.72 ng d/ml respectively) was significantly lower than that for the subcutaneous treatment (24.57 ± 4.30 ng/ml and 129.41 ± 14.81 ng d/ml respectively). No significant differences in C_{max} and AUC were found between MXD and IVM after oral administration. But after the subcutaneous treatment a greater C_{max} and AUC were obtained for MXD compared to IVM (6.12 ± 1.15 ng/ml and 59.99 ± 13.70 ng d/ml respectively) demonstrating a greater systemic availability for MXD.

For both drugs, the oral administration generated lower bioavailability than the subcutaneous route and this result deserves to be correlated with efficacy studies.

Pharmacokinetics and efficacy of ricobendazole after its subcutaneous administration to cattle at a dose of 4mg/kg

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Albendazole (ABZ), a potent member of the benzimidazolic family with wide antiparasitic spectrum, is largely used in veterinary medicine. It was reported that ABZ activity is mainly due to its metabolism to sulfoxide and sulfone. ABZ sulfoxide (ricobendazole (RBZ)) is, indeed, the drug that reach the highest plasma concentrations, being ABZ undetectable after its administration to cattle. It appears reasonable, therefore, to expect good therapeutic efficacy from RBZ. The objective of the present paper was to characterize the pharmacokinetic behaviour of RBZ after its SC administration to cattle at a dose of 4mg/kg correlating it with its anthelmintic efficacy.

Nine young parasitized castrated male Aberdeen Angus bovines weighting 150-250kg were used in the present experiment. Six of the experimental animals were injected subcutaneously with 4mg/kg ricobendazole (Sintytal R (r), Biogénesis-Sintyal, Argentina). The remaining three being kept as controls. Blood samples from the injected animals were drawn at the following post-administration hours: 2, 6, 12, 24, 48, 72 and 96. RBZ and ABZ sulfone were extracted by liquid-liquid methodology and determined by HPLC with UV detection. Three of the experimental animals were sacrificed 19 days post-administration. Parasite counts being performed in abomasum, small and large intestine looking for larvae, and after peptic digestion of abomasum mucosa looking for L4 Ostertagia adults.

There are several interesting differences to establish between orally or parenterally administered benzimidazolic agents. When ABZ is administered orally its concentrations are undetectable and high levels of RBZ and sulfone are present, this is coincident with the majority of references in this subject. In the case of parenterally administered RBZ, we found an increase of persistence of serum concentrations that permits the interpretation of a pharmacokinetic advantage that is coincident with a high anthelmintic efficacy.

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Pyriproxyfen effect on adult *Rhipicephalus sanguineus* ticks by on-animal application

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Pyriproxyfen (PPF) is an insect growth regulator developed as an ectoparasiticide for on-animal application. In this communication its effect on engorging females of *Rhipicephalus sanguineus* (Latreille) and their offspring is reported.

48 rabbits had their back shaved and were split into 8 groups of 6 each. PPF was sprayed at the following concentrations on the shaved area: 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 mcg/cm² of skin. A negative control group (no treatment) and a positive one (1mg of PPF/cm²) were included. Twenty four hours later, 20 female and 5 male ticks were put inside a closed dish stuck onto the skin of each rabbit. After engorgement and natural detachment, the female ticks were weighed, maintained for oviposition and their egg clusters weighed. 100 eggs per cluster were incubated at 90% RH and 30°C. Hatched eggs and any living larvae were counted 10 days later. One month after hatching, the remaining larvae were put on untreated rabbits and any that fed were recorded.

The results show that the post-treatment effects of PPF at 0.8 mcg/cm² and above decrease hatchability, or affect survival and feeding behavior of larvae, and therefore break the life cycle of *R. sanguineus*.

group	weight (mg) of		hatched egg (n)	larvae that (n)	
	females	eggs		survived	fed
control -	280.9 ± 20.3	180.0 ± 25.9	98.9 ± 1.8	98.2 ± 2.4	95.3 ± 1.8
control +	273.3 ± 21.9	0	-	-	-
0.1 mcg	269.3 ± 26.4	154.8 ± 23.9	98.8 ± 1.6	97.9 ± 2.1	96.3 ± 2.3
0.2 mcg	274.5 ± 28.3	158.6 ± 27.3	98.4 ± 2.2	96.4 ± 3.5	94.5 ± 3.1
0.4 mcg	268.2 ± 30.0	152.4 ± 29.7	98.0 ± 2.2	96.0 ± 2.9	93.3 ± 3.0
0.8 mcg	273.7 ± 23.8	153.1 ± 22.9	48.9 ± 4.6	46.6 ± 5.5	19.8 ± 5.6
1.6 mcg	258.8 ± 29.0	151.6 ± 26.7	37.8 ± 7.3	0	-
3.2 mcg	276.8 ± 23.7	140.5 ± 21.6	0	-	-



Integrated tick control : breaking the life cycle (1)

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Alike fleas, *Rhipicephalus sanguineus* ticks [Rs] are able to complete their life cycle in or close to human households. Therefore, concepts for flea control deserves to be checked for ticks, with the aims to kill ticks before they initiate reproduction (1) and to prevent viable reproduction (2). 30 short haired dogs were split into 3 groups of 10 : a no treatment group [CTL], a 0.02% pyriproxyfen + 2% permethrin spray group [DUO] (Duowin[®], Virbac[®], 5 ml/kg) and a 0.25% fipronil spray group [FRL] (Frontline[®], Rhône Mérieux[®], 3 ml/kg). Each dog was infested weekly with 25 females and 10 males Rs from day 7 to 77 post-treatment, ticks being counted and removed 7 days later. As the size of the blood meal determines the weight of ticks and eggs clusters, thus their ability to reproduce, the engorged females collected at each count were weighed and maintained for oviposition. Their number remained low in the treated groups compared to CTL. Yet, the residual efficacy was more prolonged in DUO than in FRL. In DUO, ticks and egg cluster weights were lower than in the other two groups but on the last count, showing a strong effect on feeding behaviour of ticks and allowing to predict a drop in their reproduction capability.

group	14	21	28	35	42	49	56	63	70	77	84
day post-treatment											
mean number of engorged ticks/dog											
DUO	0	0	0.1	0.2	0.2	0.1	0.1	0.3	1.4	1.5	1.9
FRL	0.2	0.1	0.2	0.4	0.4	0.2	0.8	5.8	8.4	10.9	12.8
CTL	19.0	18.0	18.4	18.5	19.2	20.3	18.8	18.8	19.2	19.2	18.9
mean weight of engorged ticks (mg)											
DUO			125	105	81	115	125	106	131	178	218
FRL	234	261	221	248	216	230	260	268	268	278	285
CTL	259	282	289	278	282	270	279	270	281	289	289
mean weight of egg clusters (mg)											
DUO			45	12	6	58	65	25	53	74	119
FRL	116	112	112	120	101	105	119	145	155	149	132
CTL	152	150	154	155	150	142	148	150	157	138	135

* no alive engorged ticks present on dogs

NB : many significant differences by Anova, Kruskal Wallis ($p < 0.05$)

Integrated tick control : breaking the life cycle (2)

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A first report (1) has shown that the combination of an IGR, pyriproxyfen, and an adulticidal, permethrin, was able to kill most of the pests before they initiate reproduction and that surviving ticks had a low weight and oviposit small egg clusters if any. 100 eggs per ticks collected from the dogs of 3 groups of 10 - a no treatment group [CTL], a 0.02% pyriproxyfen + 2% permethrin spray group [DUO] (Duowin[®], Virbac[®]) and a 0.25% fipronil spray group [FRL] (Frontline[®], Rhône Mérieux[®]) - were incubated at 90% RH and 30°C. Hatched eggs and alive larvae 10 days later were counted. One month after eclosion, the larvae were put on albino rabbits and those feeding were counted. Egg hatch was nil in DUO group until day 77 while present in the other groups. Larvae survival and feeding was related to egg hatch. Although some adults Rs survived despite the treatments or/and at the end of the prolonged residual effect of the treatments, the life cycle was broken by the action of the combined use of compounds with different modes of action, adulticidal insecticide and ovidical-larvicidal IGR. Therefore the second step of a new integrated tick control in pets was reached with DUO, the prevention of viable reproduction being established.

group	14	21	28	35	42	49	56	63	70	77	84
day post-treatment											
mean number of hatched egg/tick											
DUO			0	0	0	0	0	0	0	30.1	73.4
FRL	97.0	100	94.0	94.7	97.0	94.6	97.1	98.7	98.8	96.2	
CTL	99.5	98.7	98.4	99.2	98.6	98.5	99.4	98.4	97.9	95.5	94.5
mean number of surviving larvae / 100 eggs											
DUO										29.3	70.0
FRL	93.5	100	91.5	92.5	93.7	95.0	93.3	95.8	98.0	97.4	92.8
CTL	98.3	97.9	98.2	97.7	98.3	97.6	99.1	97.5	96.9	93.7	92.8
mean number of feeding larvae / 100 eggs											
DUO										27.8	67.2
FRL	90.0	96	90.0	91.5	92.7	94.0	91.7	93.6	96.3	95.1	87.4
CTL	97.1	98.6	98.8	98.2	98.9	98.2	97.8	95.9	95.4	89.1	87.8

* no alive engorged ticks present on dogs

** no egg hatch

Intestinal helminths in different population groups in Northern Namibia

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Prevalence surveys for intestinal helminths in northern Namibia were done in the Kavango region, stretching a distance of 400km along the Okavango River, in East Caprivi, and area between Zambia and Botswana, and Kaudom Game Reserve and Bushmanland, situated West and South-west of East Caprivi and West of Botswana.

In a total of 4 174 fecal specimens from hospital patients in Kavango territory, hookworm (6.8 %) was the most common helminth, followed by *Strongyloides stercoralis* (2.7 %), *Hymenolepis nana* (1 %), *Schistosoma mansoni* (1 %) and *Taenia* sp (0.9 %).

In 1986, stools from 103 school children in Bushmanland showed 85 % had hookworm, 25 % *Strongyloides stercoralis*, and 13 spurious *Physaloptera* sp. Infections were also recorded. Stools from 31 Bushmen living in the Kaudom Game Reserve, in 1988, showed 63 % hookworm and 35 % *Trichuris*.

In 1990, 4000 men, women and children of Bushman descent were translocated from West Caprivi and western Bushmanland to South Africa. Stool surveys were conducted on two separate groups of children (totals 53 and 140), the first 4 months after arrival and the second a year later. Hookworm - 48/53 (91 %) : 112/140 (80) was predominant, followed by *Strongyloides fuelleborni* - 42 (79) : 57 (41), *S. stercoralis* 25 (47) : 17 (12), *Trichuris* - 1 (2) : 2 (< 2), *Taenia* sp. 1 (2) : 1 (< 1), *Hymenolepis nana* - 0 : 1 (< 1), *Ascaris* - 0 : 1 (< 1); and 24 (57) : 13 (23) harboured both strongylid species, respectively.

Stools cultured in Bushmanland and examined 24-72 hours later, contained free-living adult *Strongyloides fuelleborni* and rhabditoid larvae of *S. stercoralis*. Chinese hamsters (*Cricetus griseus*) fed filariform (F3) hookworm larvae per os and killed > 60de later, revealed adult *Necator americanus*. No cases with *Ascaris* were found in Kavango, Bushmanland or KOR.

In baseline surveys at 8 schools along the Cuando River of East Caprivi, *S. mansoni* prevalences ranged from 63-96 %, hookworm 7-58 %, followed by *Strongyloides stercoralis* 0.6-26 %, while *Ascaris lumbricoides*, 0-1 % and *Trichuris trichiura* 0-0.4 % were rare.

Evaluation of an evasive method for the control of parasitic gastroenteritis in dairy calves in the Netherlands.

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The epidemiological pattern of parasitic gastroenteritis (PGI) in dairy calves in West Europe includes the following: calves infect themselves with overwintered larvae of gastrointestinal nematodes immediately after turnout; patency starts three weeks later. As development of the free-living stages is slow in spring high pasture infectivity levels cannot be expected before the middle of July. Therefore control of PGI may be achieved by repeated moves of calves to clean pastures from July onwards. This was tested in two trials with a grazing season from May to October, using faecal egg counts, pasture larval counts, serum pepsinogen values, serology with a specific recombinant *Cooperia oncophora* antigen, weight gain, tracer worm counts and worm counts as parameters.

In both experiments initial overwintered infections were low. High infections developed in the groups which were moved once (July) or twice (July, August). Low infections were maintained until the end of the grazing season in groups which were moved three times (July, August, September). Challenge infections at the end of the second experiment indicated development of equal levels of immunity in all three groups on pasture.



An ELISA with a recombinant *Cooperia oncophora* antigen may be used for herd health monitoring of gastrointestinal nematode infections in young dairy cattle.

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The ELISA, using crude worm antigens of *Ostertagia ostertagi* and *Cooperia oncophora* has been used successfully in epidemiological studies on parasitic gastroenteritis (Ploeger, thesis 1989). However, for routine monitoring of parasitic infections higher specificity and better standardisation are required than is possible with crude worm antigens. Therefore, we tried to develop test with a more suitable antigen for *C. oncophora*. This test should have a high specificity and it should be possible to discriminate between different exposure levels in young cattle.

A recombinant 14.2 kDa *C. oncophora* protein met all these requirements. Competitive ELISA demonstrated that it contained no epitopes shared by the other major gastrointestinal nematodes of cattle or even *C. curticei*. Tested in an ELISA during and after the first grazing season in over 20 experimental groups of calves with well known natural infections it showed a correlation with exposure levels.

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Is the periparturient rise in faecal egg count the only significant contribution of the ewe to pasture larval contamination?

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The South Island of New Zealand is a temperate winter rainfall zone with temperatures frequently below zero and with occasional snow during the winter. From 1982 the faecal egg count (FEC) of pasture fed ewes has been monitored on a regular basis. Significant rises in FEC of pregnant ewes have been frequently observed during early to mid-pregnancy, in which case the typical periparturient rise does not occur. The effect is generally more apparent in younger ewes. Pasture larval populations are frequently in excess of 2000 larvae/kg pasture WMB during the winter months of June and July (predominantly *Teladorsagia* [*Ostertagia*] *circumcincta* and *Trichostrongylus colubriformis*). Calculations indicate that ewes, at times, consume between 20,000 and 30,000 larvae per day. Considerable development of eggs and larvae takes place throughout the winter months with L³ of both species developing within 30 days. These effects suggest pasture larval populations in the spring arise from both multiplication of autumn infection through ewes and winter development as well as carryover of larvae from autumn contamination. The use of slow release devices (CRCs containing albendazole) during the autumn and winter in ewes which resulted in 11% greater weight of lambs weaned per hectare ($p < 0.05$), tend to confirm these conclusions.

The anthelmintic efficacy of reduced dose copper oxide wire particles in sheep and deer.

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It has been found that the administration of 5g copper oxide wire particles (COWP) to sheep have anthelmintic efficacies of 96% and 56% against *Haemonchus contortus* and *Teladorsagia* (*Ostertagia*) *circumcincta* respectively. In view of the increasing problem of anthelmintic resistance, the evaluation of alternative methods of control need to be examined but this has to be examined in light of any possible toxic effects. Trials were undertaken to examine the effect of 2.5g COWP on 7 month old sheep with established *H. contortus* infections and on incoming *H. contortus* L3 larvae. It was found that 2.5g COWP had a 97.4% efficacy against established populations and 98.5% against incoming L3. No significant differences in liver copper levels were found between control and COWP treated animals at the end of the trial because the diet contained 25ppm copper. Copper supplementation trials involving pasture fed red deer (*Cervus elaphus*) showed a significant ($p < 0.05$) weight response in COWP treated yearling stags when compared to both control and copper EDTA injection treated animals despite the fact that both copper treatments raised liver copper to similar levels. Average serum pepsinogen levels were less for the COWP treated animals (not significant) than for the other two groups despite regular benzimidazole treatment. Production responses attributed to copper supplementation involving COWP treatment in ruminant animals should allow for the possible anthelmintic effect of copper. However efficacy may be reduced when abomasal pH is >3.5 as may be the case with *T. circumcincta* infection when dissolution of the COWP may be reduced.

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Persistent efficacy of doramectin pour-on against *Haematobia irritans* in cattle

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Four studies were conducted under field conditions in Hungary and Germany to determine the persistent efficacy of doramectin administered topically in controlling horn fly (*Haematobia irritans*) infestations on cattle.

In each study, 39 to 41 cattle with naturally acquired *H. irritans* infestations were randomly allocated in a tiered manner to a doramectin treated or a non-medicated control group (19 to 21 animals in each) on the basis of body weight. Each animal in the treated groups received doramectin at a dosage of 500 µg/kg (1 mL/10 kg) on day 0. Horn fly counts were carried out on all the animals prior to treatment and at weekly intervals thereafter until the end of the studies on day 49.

Very few *H. irritans* were observed on cattle in the treated groups from day 7 after treatment until the end of the study, and the horn fly counts were significantly lower ($P=0.0001$) compared with those of non-medicated animals at all times in 3 studies. In the fourth study, numbers of horn flies on the non-medicated cattle were too low after day 28 to allow a meaningful estimate of efficacy. Excluding this last study, percentage reductions in horn fly counts of the doramectin-treated animals were 93.4%, 92.8% and 75.1% on days 35, 42 and 49 respectively. No adverse reaction to treatment was observed at any time during the studies. In two studies doramectin-treated cattle had a significantly greater ($P=0.001$ and $P=0.012$) daily weight gain during the 49-day study period than the animals in the non-medicated group.

A single application of doramectin pour-on, administered to cattle at a dosage of 500 µg/kg was highly effective in reducing horn fly infestations for at least 42 days after treatment.

Molecular analyses of *Tritrichomonas foetus* and related flagellates

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The taxonomic situation in the genus *Tritrichomonas* is the subject of controversy. *T. foetus* and *T. suis*, the tritrichomonads from cattle and swine, could potentially belong to the same species. This question was tackled by a molecular biological approach: The 5.8S rRNA gene and the flanking internal transcribed spacer regions (ITS1 and ITS2) of twelve different isolates of the three *Tritrichomonas* species *T. foetus*, *T. suis*, and *T. mobilensis* were amplified by PCR and subcloned. Other trichomonads (*Trichomonas vaginalis*, *T. tenax*, *T. gallinae*, and *Pentatrichomonas hominis*) were included for comparison. Sequence analysis of cloned fragments was performed. In contrast to the genus *Trichomonas* where more diversity was observed, the genus *Tritrichomonas* showed an extremely high degree of homogeneity. All *T. foetus* and *T. suis* isolates had identical sequences, only one substitution was found in the ITS2 region of *T. mobilensis*. These results would support a future revision of the taxonomic classification of tritrichomonads.

Detection of *Tritrichomonas foetus* by polymerase chain reaction (PCR) and DNA enzyme immuno assay (DEIA) based upon rDNA sequences

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Tritrichomonas foetus is the causative agent of bovine tritrichomonosis, a sexually transmitted disease leading to infertility and abortion in cattle. Diagnosis is hampered by frequent contamination of samples with intestinal or coprophilic flagellated protozoa which might be mistaken for *T. foetus*. Therefore, a polymerase chain reaction (PCR) test optimized for applicability in routine diagnosis was developed. Amplification is based upon primers TFR3 and TFR4 delineated from the rDNA gene units of *T. foetus*. In order to avoid potential carry-over contaminations by products of previous amplification reactions, conditions were established for the utilization of the uracil DNA glycosylase (UDG) system. Furthermore, documentation and interpretation of results were facilitated by including a DNA enzyme immuno assay (DEIA) for detection of amplification products. The high specificity was confirmed with genomic material from different related trichomonadid protozoa. The sensitivity allowed to detect about 50 *T. foetus* parasites in preputial washings. The PCR was assessed comparatively with the Tf-InPouch® test upon 100 diagnostic samples from bulls/cows in Switzerland and upon 7 putative cases of trichomoniasis in bulls under quarantine. The methods proposed are highly suitable as a confirmation test of microscopical diagnosis after *in vitro* cultivation, and as well as a direct screening test for *T. foetus* in infected body fluids.

FUNCTIONAL MORPHOLOGY OF THE GNATHOSOMA OF *Raillietia flechtmanni* (Acari:Gamasida)

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The gnathosoma is a small anterior trophic-sensory region of the body of mesostigmatic mites. This structure is composed by: palps, hypostome, labrum, salivary ducts, mouth and chelicerae. Despite its apparent simplicity, the gnathosoma represents a highly evolved and specialized body region of mites. Members of the mesostigmatic sub-order Gamasina exhibit a wide variety of feeding habits and this diversity in feeding is reflected in the structure of the gnathosoma. *Raillietia flechtmanni* is an important etiologic agent of the bovine otocariasis in Brazil. Until the present time the structure of the gnathosoma of the *R. flechtmanni* were unknown, particularly their histology, ultrastructure and functional morphology.

In this work, female of *R. flechtmanni* was collected from ear canal of cattle, fixed in glutaraldehyde 2.5%, pH 7.2, 0.1M, processed and included in resin in order to obtain serial sections of the gnathosoma about 1 µm thickness. This sections were stained with toluidine blue 1%. Some mites were prepared for scanning electron microscopy (S.E.M.) according Ferry et al. 1994.

The structure of the sclerotized parts of the gnathosoma: hypostome, labrum, palps and chelicerae; the muscles, nerves, pharyngeal and salivary conducts, were studied in histological sections, and compared with the S.E.M. observations, supporting an hypothesis to explain their functional morphology, feeding mechanism and their role on the pathogeny of the lesions in the external ear meatus of the hosts.

The histological and S.E.M. studies of the *R. flechtmanni* gnathosoma shows that the chelicerae has two horn-like structures distally in its body: the fixed digit and movable digit, that make contact like a tweezers and probably holding the "food" and contribute to make damage in the ear epithelia. The retraction of the chelicerae lead the "food" to dorsal region of the hypostome where the salivary stilus can be seen. In this region the salivary secretion make a pre-digestion of the "food" and then the "food" are swallowed by a sucking movement determinate by the antagonic action of the dilator and the constrictor pharyngeal muscles.

Comparative morphology of the gnathosoma of *Raillietia auris*, *R. caprae* and *R. flechtmanni* (Acari:Gamasida) under scanning electron microscopy.

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There are seven described species of *Raillietia* Trouessart. Three of them, *R. auris* Trouessart, 1902; *R. caprae* Quintero, Bassols and Acevedo, 1980; and *R. flechtmanni* Faccini, Leite and Costa, 1992; have been recorded from the ear canals of cattle, goats and sheep, and buffalo and cattle, respectively in Brazil (Faccini et al. 1992 - Rev. Bras. Parasitol. Vet. 1: 109-110). Research carried out in the last decade in Brazil (Leite et al. 1989a - Arq. Univ. Fed. Rur. Rio de J., 12:83-91) and elsewhere (Jubb et al. 1993 - Aust. Vet. J., 70:354), has shown that these mites are pathogenic although the economic importance of the parasitism is still unknown.

The mites used in this study were obtained from the external ear canals of cattle, buffalo and goats from the city of the Rio de Janeiro according to the methods described by Leite et al. 1989b (Mem. Inst. Oswaldo Cruz, 84:309-311), and then prepared from scanning electron microscopy according standard methods described by Ferry et al. 1994. (Rev. Bras. Parasitol. Vet. 3:65-68)

In this work some morphological characters which distinguish the gnathosoma of the three species of *Raillietia* in Brazil were studied under S.E.M. Morphological differences were noticed in the movable digits of chelicerae of the male and in the shape of the gnathotectum of the male and female, the remaining structures were similar to each other. The movable digit of the male has a complex morphology characterized by the presence of two filamentous and a third smaller, digitiform projection, fused to form the spermatodactyle in *R. auris* and *R. flechtmanni*. In *R. auris* the ventral basis of the larger projection resembles a bulb. In *R. caprae* the projections are shorter and spine-like.

Under S.E.M. the gnathotectum is an elaborated fringe structure, dorsally convex whereas the ventral surface is concave. Noticeable differences were observed in the shape among the three species: in *R. auris* the gnathotectum has a isosceles triangle shape, in *R. flechtmanni* a pentagonal shape and in *R. caprae* it is rounded. Concerning *R. caprae*, our observations are in accordance with Domrow, 1979/80 (Proc. Linn. Soc. N.S.W. 86:60-95) and Quintero et al. 1980 (Vet. Mexico 11: 17-20).



A field study to examine the effects of twice weekly dung collection from pasture on the strongyle ERP (egg reappearance period) in the faeces of yearling ponies.

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Two groups, each of nine yearling Welsh Mountain ponies, grazed from May to the beginning of November 1996 on two separate, equal sized, paddocks. Each paddock had a similar history and both had been grazed by ponies since February. On day 0 the ponies were allocated to group on the basis on sex and faecal egg-count. All ponies were treated with ivermectin (Eqvalan, MSD AgVet) on Day 0 and retreated whenever their arithmetic group mean faecal egg-count exceeded 200epg and at least 25% of the group were positive. Faecal egg-counts were performed at fortnightly intervals and pasture samples (from roughs, lawns and the whole pasture) were collected at monthly intervals. Dung was collected manually from one paddock twice weekly throughout the study, while dung was left in the second paddock. Both groups of ponies met the retreatment criteria on days 56 and 140 of the study, when all ponies were retreated with ivermectin (Eqvalan, MSD AgVet). Pasture larval counts were zero until day 56 (12th July) when 80 larvae per kilo wet matter (Kg WM) of grass were recovered from the whole pasture sample, dung collected field. Peak pasture larval counts occurred on the dung collected field on day 112 (6th September) with 520 larvae per Kg WM from the lawn samples. Peak larval recovery from the non-dung collected pasture was seen on day 140 (4th October) when 3458 larvae per Kg WM were retrieved from the rough sample, the lawn sample from the same day carried 1003 larvae per Kg WM of grass. Thereafter pasture larval counts diminished rapidly in both paddocks. While twice weekly dung collection failed to alter the retreatment interval, it resulted in a marked reduction in larval contamination of pasture in the autumn.

EPIDEMIOLOGY AND CONTROL OF TICKS AND FLEAS ON PETS IN SOUTH AFRICA

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The objectives of this study were to provide information on the ticks infesting dogs, tick related diseases, the ratio of different flea species that infest dogs in various geographical areas in South Africa and also control measures practised. Information on flea infestations of cats are also given. Twenty nine different ixodid tick species, which belong to 8 different genera, are known to parasitize dogs in South Africa. This constitutes almost 35% of the ixodid ticks already described in South Africa. Dogs are considered the preferred host of three of these tick species, namely, *Haemaphysalis leachi*, *Rhipicephalus sanguineus* and *Rhipicephalus simus*. Fifteen species of ticks collected from dogs are considered to be accidental infestations. Tick related diseases observed in dogs include canine ehrlichiosis, canine babesiosis, canine hepatozoonosis, toxicosis, including paralysis and the formation of large necrotic lesions, and blood loss anaemia. The fleas which commonly infest dogs in South Africa are *Ctenocephalides felis* spp. and, in per-urban and rural environments, also *C. canis* and *Echidnophaga gallinacea*. Pet owners residing in affluent areas use various commercially available chemicals to control ticks and fleas on their dogs whereas those in resource poor environments use home made products such as mixtures of Jeyes fluid (disinfectant) and used engine oil or water.

Infection with *Trichinella* in farmed crocodiles (*Crocodylus niloticus*) in Zimbabwe

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A total of 648 crocodiles from 17 farms, in different areas of Zimbabwe, were examined histologically for *Trichinella* infection during the period 1995/96. *Trichinella* was present on eleven farms and larvae were demonstrated in 49 of the 421 crocodiles examined on these farms, while *Trichinella* "nurse cells" only were seen in a further 107 crocodiles. The prevalence of infection was therefore 37.1 %, compared with a prevalence of 39.5 % when pepsin digestion of the muscles was done. Between 1 and 37 viable larvae g⁻¹ were recovered, mainly from pterygoid, mandibular and intercostal muscles. The parasite was identified as *Trichinella spiralis* on morphological features.

Rats fed fresh crocodile meat containing 13 *Trichinella* larvae g⁻¹ became infected eight weeks later. Larvae were concentrated mainly in masseter, pterygoid and intercostal muscles. Two baboons were drenched with an homogenate of *Trichinella* infected crocodile meat. One of them was examined at post mortem, eight weeks later, and between 17 and 405 *Trichinella* larvae g⁻¹ were recovered from 14 different muscles. Laboratory-reared crocodiles fed meat from this baboon were sacrificed eight weeks later and were shown to be infected with *Trichinella*.

Crocodiles infected with *Trichinella* in the laboratory were twice dosed orally with a 7.5 % suspension of albendazole, administered at a dose rate of 50mg kg⁻¹, at an interval of 4 days. No larvae could be recovered from two of these crocodiles which were sacrificed while only 1 larvae g⁻¹ was present in the pterygoid muscle of a third. The muscles of untreated, control crocodiles contained 1-16 larvae g⁻¹. Rats were fed between 8 to 60g of *Trichinella*-infected crocodile meat which had been frozen at minus 18°C for 7, 14 and 21 days. No parasite larvae were found in their muscles. Rats fed unfrozen meat from the same source were infected with 116-513 larvae g⁻¹.

This is the first report of trichinellosis in crocodiles, or in any reptile, and presents a new epidemiological entity and potential zoonotic problem because crocodile meat is sold for human consumption. These new data indicate a need for research on the origin of *Trichinella* in crocodiles as well as the epidemiology, pathogenesis, immunology and control of infection with respect of the husbandry of farmed crocodiles.

Control of biting lice with triflumuron

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Biting lice of the genera *Bovicola* and *Damalinea* pose a problem of considerable magnitude to small stock farmers in South Africa. *B. ovis* infestations in sheep are increasing whereas *Damalinea limbata* infestations in Angora goats currently occur very wide spread. Failures to control lice with organophosphate and synthetic pyrethroid insecticides are reported regularly, suggesting that resistance to these chemicals is wide spread. The use of a chemical with an alternative mode of action is required since a complete breakdown in the control of lice infestations can be ill afforded. In this study the efficacy of Triflumuron, an insect growth regulator developed from a substituted benzoylphenylurea group, to control lice infestations on sheep and goats, were investigated. Both small scale field studies employing untreated control animals, and large scale field studies where the whole flock was treated, were conducted. Zapp (480g/L Triflumuron) at concentrations ranging from 0.52-15ml/L water were used as a submersion dip to treat animals. Lice were counted at regular intervals during the studies which ranged from 12-20 weeks. Within two weeks in excess of 80 % of nymphs on the animals were killed. It took up to eight weeks before no lice could be observed in Angora goats and in the case of Merino sheep 12-14 weeks. Triflumuron is very effective to control biting lice on sheep and goats.



Sheep scab : effect of breed on population growth

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All indications are that sheep scab is spreading at an alarming rate in South Africa. Surveys conducted in the Free State have shown that the symptoms of the disease is not as apparent in Dorper compared to Merino sheep. It was hypothesised that due to this phenomenon Dorper sheep may be important in the spreading of the disease. Comparative studies on Merino and Dorper sheep were conducted and increase in lesion size was used as an indicator of population growth. Ten Merino and Dorper sheep each were infested with *Psoroptes ovis* mites on three places on their backs. The surface area of the scab lesions were determined fortnightly over a period of six weeks. Sheep scab lesions increased at a significantly faster rate on Merino compared to Dorper sheep. The total surface area of scab lesions on Merino were almost five times greater, compared to Dorper sheep. The role played by Dorper sheep in the epidemiology of sheep scab should be further investigated.

Antifeeding effect of several insecticidal formulations against *Ctenocephalides felis* in cats

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This study was designed to evaluate the biting ability of fleas following various insecticidal treatments in cats.

30 cats were allotted to 6 groups of 5. Each cat was housed in a separate cage. At Day 0, each group of cats received a single treatment : - Group 1 : spot-on application of imidacloprid (Advantage®) cats < 4kg : 40mg/cat, cats ≥ 4kg : 80mg/cat; - Group 2 : spot-on application of fipronil (Frontline spot-on®) 50mg/cat; - Group 3 : spray application of fipronil (Frontline spray®) 7.5mg/kg b.w.; - Group 4 : moussé application of permethrin (Defencat ®) 50mg/kg b.w.; - Group 5 : aerosol spray application of dichlorvos + fenitrothion (Nuvan Top ®), 1 second/kg b.w. and - Group 6 : control group : cats were not treated.

One hour after the treatment, each cat was infested with 50 unengorged young adult fleas *Ctenocephalides felis* deposited along the dorsal midline. One hour later, each cat was carefully combed using a fine-toothed comb (13 teeth/cm). Collected fleas were swatted to detect blood in their abdomen. Reinfestations were performed over the claimed persistency of the tested products : at days 3, 7 and 14 for all groups; at days 21 and 30 for groups 1-2-4-6; at days 35 and 42 for groups 3-6. Cats were combed one hour after each reinfestation.

	1h	D3	D7	D14	D21	D30	D35	D42
Gr 1	24.8±6	15.5±5	15.2±6	26.8±11	27±8	32.6±3		
Gr 2	16.8±2	18.6±6	15.2±6	18.2±4	24.2±4	28.4±3		
Gr 3	18.4±3	19.2±5	15.8±2	19.2±2	24±5	28.4±5	29.2±4	30.4±3
Gr 4	1.2±1	0.4±1	2.2±3	19.4±3	28.2±2	31.6±3		
Gr 5	3.6±1	5.4±4	18.4±5	27.4±6				
Gr 6	25.4±5	29.4±3	22.2±2	28.6±4	30.6±4	29.8±3	29.8±4	29.6±4

It is concluded that the topical application of imidacloprid or fipronil does not prevent fleas from biting and feeding during the first hour after infestation prior to being killed. The topical application of dichlorvos/fenitrothion and permethrin leads to a significant decrease of the number of engorged fleas for 3 and 7 days post treatment respectively.

Proteases in nematode infection and development

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Nematode parasites are challenged with a variety of obstacles during their life history including survival in the external environment, passage of various host barriers and survival under adverse host conditions. In addition, they must undergo a series of complex developmental events and acquire sufficient nutrition to maintain homeostasis in the host, grow and reproduce. Proteases have been demonstrated to play key roles in many of these processes. Eggs of *Haemonchus contortus*, a nematode parasite found in the abomasum of sheep, are passed in feces onto pastures where they develop to infective larvae. Proteases have been demonstrated to assist the first-stage larvae in hatching by softening the shell prior to mechanical breakage. Free-living, infective third-stage larvae, bearing the second molt cuticle, must exsheath rapidly upon entry into the host. A metalloprotease secreted by the parasite causes rapid digestion of a specific area of the sheath (based on a unique hydrolytic site) allowing the parasite to escape within minutes following ingestion. During the early stages of development, fourth-stage *H. contortus* larvae secrete another metalloprotease, which appears coincident with the initiation of feeding. This enzyme degrades a variety of substrates but with greater specificity for fibrinogen; the role of this protease may include exsheathment and nutrient acquisition. Adult *H. contortus* produce a variety of proteases, notably a family of cysteine proteases. Although the exact role of these proteases is uncertain, secreted cathepsin L-like proteases were shown to degrade an extracellular matrix resulting in uptake of released products by the parasite. In addition, both cysteine and aspartic proteases have been implicated in the degradation of albumin by adult *H. contortus*.

Evaluation of helminth infections in tarpans using the method of collection worms after the treatment

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Tarpans (*Equus caballus* Gmelini Ant.) are reconstructed breed of wild horses which had lived till the end of XIX century on the steppes of the West of Ukraina and Eastern part of Poland. The study was performed to evaluate intensity of helminths infection in stabled tarpans in the Reserve of Popielno (North part of Poland, Region of Great Mazurian Lakes). Four naturally infected tarpans after faecal samples examination were chosen to the experiment. Two of them were dosed with Eqvalan-paste (MSD-Agvet) and two were left as an untreated control. 24 hours after dosing within 3 days all the faeces was collected. In two control tarpans no parasites were found. 14 species of nematodes and one of botfly larvae were recovered from the faeces of treated animals. The nematode species included 12 cyathostomes (small strongyles), *Strongylus (Alfortia) edentatus* (large strongyle) and *Oxyuris equi*. The number of cyathostomes species were 12 and 8 in two treated horses and the total numbers were 1422 and 73 Cyathostominae. In the order of abundance cyathostomes species found were : *Cyathostomum catinatum*, *C. pateratum*, *Cylicostephanus longibursatus*, *Cylicocycylus nassatus*, *Cyathostomum coronatum*, *Cylicocycylus triramusus*, *Cylicostephanus minutus*, *C. goldi*, *C. calicatus*, *Cyathostomum labiatum*, *C. labratum* and *Cylicocycylus leptostomus*. The most abundant species *C. catinatum* made up 72.4 % of the total cyathostomes in horses examined. These results are compared with the earlier surveys based on autopsies of working horses in Poland and studies in other countries. In Popielno reserve tarpans have been living more than 40 years in isolation on a peninsula. Present results confirm the stability of cyathostomes in different breed of horses over the world.



Evaluation of Aversectin efficacy, a new broadspectrum antiparasitic drug in horses

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Aversectin oral paste formulation for horses is a new drug from avermectin-group which has been introduced in the market in Russia since 1996. A preliminary study in a Stud of Ukraina in 1996 has shown 100% efficacy of Aversectin against horse strongyles and ascarids during four weeks after dosing. The current study was conducted in Poland to evaluate a long term effect of the drug, and to compare its effectiveness to two other anthelmintics commonly used: Eqvalan (ivermectin) and Telmin (mebendazole). Twenty-seven standardbred adult horses from Pleasure Horse Stable with strongyle fecal egg counts ranging from 100 to 2550, with a mean of 668 EPG were used. Six of them were stated to be infected with ascarids. The horses were divided into three groups of nine animal each, receiving Aversectin (0.2 mg/kg), Eqvalan (0.2 mg/kg) and Telmin (10 mg/kg). Treatment occurred in September and fecal samples were examined by McMaster technique every four week till January. No ascarids eggs were found till the end of the study. Four weeks after treatment no strongyle eggs were found. After eight weeks a mean EPG level in Aversectin, Eqvalan and Telmin treated group was 64, 17 and 119 respectively. EPG increase was observed after 12 weeks of the study and 236, 118 and 261 eggs per gram in three groups of horses were found. Four months post treatment in January, the mean EPG level for Aversectin, Eqvalan and Telmin treated group was 314, 255 and 489 respectively. The results of this trial demonstrate a comparable efficacy of Aversectin to Eqvalan, both of the same chemical group. Aversectin has more prolonged effectiveness as Telmin, benzimidazole group.

Serratospiculum from falcons of the Arabian Peninsula. L. M. Gibbons¹, J. Samour² and T. Bailey²

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Specimens of an unidentified species of *Serratospiculum* Skrjabin, 1915 (Nematoda, Diplostriaenoidea), were recovered from the air sacs of four saker falcons, *Falco cherrug* by endoscopy in the United Arab Emirates. The saker falcon, the largest of the desert falcons, has been a favourite for centuries amongst the Arabs for the skilful art of falconry because of its endurance in flight, great strength and ability to cope with the rigorous environment of the Arabian Peninsula.

Serratospiculum is probably the single most important parasite in falcons in the region occurring in both the lungs and airsacs and its specific identity needed to be confirmed prior to further studies on this parasite. Adult female worms measure up to 25cm long and heavy infections result in the poor flying performance of the host and laborious breathing. Heavy infections may be found in association with aspergillosis.

There are two very closely related species, namely, *S. chungii* Hoeppli & Hsu, 1929 and *S. seurati* Bain & Mawson, 1981 primarily separated by the morphology of the epaulettes on the cephalic region. The epaulettes are straight in relation to the dorso-ventral axis in *S. chungii* and concave in *S. seurati*. Using this character the specimens recovered from the Arabian Peninsula are assigned to *S. seurati*. This parasite has previously been recorded from falcons in North Africa and a subspecies of saker falcon, *F. cherrug cherrug* migrates through Northeast Africa in winter. This finding represents a new locality record for *S. seurati*.

Extension of the prophylactic effect of isometamidium in cattle using sustained release devices : laboratory and field trials

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Biodegradable poly (D,L-lactide) sustained release devices (SRD) containing isometamidium were first evaluated in cattle under controlled conditions (monthly challenge with tsetse flies infected with *Trypanosoma congolense* clone IL 1180). It was shown that subcutaneous implantation of isometamidium-SRD (at 0.5mg/kg) extended the prophylactic period by a factor 3.2 in comparison with intramuscular (i.m.) injection of the same dose of the drug. Analysis of the isometamidium concentration in the sera of the treated animals (using ELISA) showed that the drug remained present for at least 5 months at concentrations between 0.4 and 0.8ng/ml in the SRD-treated cattle, whereas in the i.m. treated group the isometamidium concentration dropped to 0.1ng/ml by 85 days post treatment.

A field trial was later carried out on a ranch in Mali where a heavy tsetse challenge was present. Two groups of about 40 cattle were treated with isometamidium (1mg/kg), either as SRD or as i.m. injection, and compared with a control group. Eight months after treatment the cumulative percentage of trypanosome infections was 28, 59 and 77 %, respectively in the SRD-implanted, the i.m. treated and the control groups. Statistical analysis showed that the period of prophylaxis was extended by at least a factor of 2 in the SRD-implanted group compared with the conventionally treated animals.

Helminth infections in goats on mixed farms in Central Kenya

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A survey was undertaken to study the epidemiology and intensity of nematode infection among goats on eight randomly selected mixed farms in a coffee marginal area of central Kenya.

Various age groups of goats were eartagged and faecal sampled at the beginning of the study. They were then treated with albendazole 10 % at manufacturers recommended dose. These goats were faecal sampled fortnightly. Weights and blood for PCV and serum albumin were taken monthly.

The intensity of infection varied with age groups of the goats and the individual farms.

Fourteen days after treatment, the faecal egg counts were reduced to zero except in one farm. Coprocultures revealed that *Haemonchus contortus* was the main nematode species infecting these goats. *Cooperia curticei* occurred in low numbers in a few farms.

Plasma nitric oxide levels in dogs infected with *Babesia canis*

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Nitric oxide and its radicals have been found to be key mediators in the pathology and control of hemoprotozoan infections. The aim of this study was to investigate plasma nitric oxide levels in dogs infected with *B. canis* and to correlate nitric oxide concentrations with disease severity. Blood samples were collected from dogs in a peri-urban area north of Johannesburg. Plasma levels of reactive nitrogen intermediates were measured using the Griess assay. A severity-of-disease classification system to standardize severity of disease was devised and dogs were stratified into uninfected (n=15), mild (n=8), moderate (n=6) and severe (n=5). There was a wide distribution of nitric oxide concentrations within each clinical category; however, there was a clear trend towards levels being elevated in the mild (mean = 64.7 µM) and moderate (mean = 51.89 µM) disease groups relative to the controls (mean = 34.93 µM) and severe (mean = 20.71 µM) disease group. Plasma nitric oxide levels were found to correlate with haematological disturbances, as assessed by haematocrit levels ($r = 0.7, p < 0.001$). There was no relationship between parasitaemia and nitric oxide levels. High levels of nitric oxide are expected to exhibit antimicrobial activity as well as suppress lymphocyte proliferation. It is postulated that nitric oxide plays a protective role in *B. canis* infections by decreasing the immune mediated hemolytic anemia.

Field trials in New Zealand demonstrating the anthelmintic efficacy of a topical formulation of eprinomectin in Red Deer

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A topical formulation of eprinomectin, a second generation avermectin/milbemycin, has been developed for use in cattle and deer. Five field trials were conducted to evaluate the anthelmintic efficacy and acceptability of eprinomectin in deer under field conditions. The studies included 289 male and female deer ranging from 5 months to 9.5 years, weighing 22.0 to 113.6kg. In one study most of the deer were 2.5 to 4.5 months pregnant on the day of treatment. In each study, deer were allocated to replicates of five animals from within a ranking on body weight within sex. In one study, the animals were also grouped by age. In each replicate, one animal was randomly allocated to a untreated control group and the other four each received eprinomectin applied topically at 500mcg/kg. Faecal samples were collected from each animal before treatment and again on approximately Day 14 for nematode egg and larval counts. *Dictyocaulus viviparus* larvae were found in four of the five studies with reductions of 98 - 100 % in faecal larval per gram counts in four studies. In the fifth study only two untreated control deer were positive on Day 0 and none were positive on Day 14. Strongylid eggs were found in all five studies with reductions of 95 - 100 % in the egg counts in four of the five studies. In the fifth study too few control animals were infected to allow a meaningful calculation of efficacy. Strongylid eggs were detected in only one of seven control animals sampled before treatment and two of the nine control animals on Day 14. Treatment with eprinomectin was well accepted with no adverse reactions to treatment. The results of these studies demonstrate the acceptability and high activity of eprinomectin against strongyle parasites and lungworm when applied under field conditions.

The safety and anthelmintic efficacy of a topical formulation of eprinomectin in Red Deer

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Eprinomectin is a second generation endectocide of the avermectin/milbemycin class, which was developed for its wide spectrum and high activity, extraordinary safety and its high plasma/milk partition coefficient in cattle. Due to the importance of Red Deer farming in some parts of the world and the preference for using topical formulations in this species, we examined the safety and anthelmintic activity of a topical formulation of eprinomectin at 500mg/kg in Red Deer in New Zealand. Eprinomectin was shown to be safe at 5 times the recommended dose rate, the highest dose tested. The therapeutic activity of eprinomectin was evaluated in a pen trial against natural helminth infection in weaned deer. Deer treated with eprinomectin had significantly ($p < 0.05$) fewer of the following parasites than vehicle-treated controls: *Dictyocaulus viviparus* (adult), *Oesophagostomum* spp. (adult), *Ostertagia* spp. (adult & L4) and *Trichostrongylus* spp. (adult & L4). The therapeutic activity of eprinomectin against induced infection with *D. viviparus* was evaluated by treating deer when the nematodes were expected to be at the fourth larval stage. Deer treated with eprinomectin had significantly ($p < 0.01$) fewer *D. viviparus* than untreated controls. The results of these studies demonstrate the safety and high activity of eprinomectin against the major pathogenic nematodes of Red Deer in New Zealand.

Molecular (PCR) and Immunological diagnosis and prevalence determination of bovine neosporosis and toxoplasmosis.

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Two PCR tests for the identification of *Neospora* sp. and *Toxoplasma gondii* have been optimized and assessed in view of their practicability for routine diagnostic application. In both PCR tests, a subsequent DNA-hybridization immuno-assay (DIA) allowed highly sensitive and specific detection of respective DNA amplification products. The diagnostic tools were validated upon experimentally infected cows. For field evaluation, we examined 83 brain tissues from aborted bovine fetuses. In 24 brains (29%), we were able to identify *Neospora*-DNA by PCR. In another 3 brains, we were finding *Toxoplasma* DNA (B1-gene-PCR). From the *Neospora*-PCR-positive fetuses, 6 were sero-positive by IFAT and ELISA. The *Toxoplasma*-PCR-positive fetuses were seronegative for P30-ELISA and in the direct agglutination (DA) test. *Neospora*-PCR-positive brains frequently showed multifocal necrotic and calcific microlesions compatible with neosporosis, whereas most of the fetuses negative in *Neospora*-PCR showed different or atypical pathological and histopathological characteristics. For serology the *Neospora*-ELISA was compared with the *Toxoplasma*-P30-ELISA using 1,689 sera originating from a dairy cattle health study in Switzerland. Anti-*Neospora* antibodies were detected in 175 (10.4%) cows (all negative for P30-ELISA) and 161 (9.5%) had anti-P30 antibodies (and were negative in the *Neospora*-ELISA). There were only 19 additional double positive animals (*Neospora* and P30 positive), less than expected statistically. A similar study was done with animals from cow-calf-operation units. Here, seropositivity in the *Neospora*-ELISA was 11% (cows) and 14% (calves) and that for the P30-antigen 14% (cows) and 15% (calves) no double positives were seen in this group.



Molecular and sero-epidemiological investigations on *Trichinella* sp. in Switzerland

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Swiss domestic pigs have been considered as *Trichinella*-free for decades, despite that *Trichinella* occurs in a wildlife cycle. In order to reevaluate the present epidemiological situation, a pilot survey study including 11'226 domestic pigs, 356 wild boars and 452 foxes were examined for *Trichinella* infestation with a standardized artificial digestion method in accordance with prevailing EC directives and simultaneously with a serological procedure (ELISA with E/S antigens). The study was extended by a retrospective serological study on 25'239 sera from sows provided by a representative Swiss pig serum bank. Among the domestic pigs, the digestion method all yielded negative results; serologically, 3 fattener pigs (0.027%) and 9 sows (0.036%) showed weak antibody reactions to the E/S-antigen. Based on statistical calculations for the determination of the test's threshold value, these findings were considered as being within the expected norm. Therefore, subject to statistical restrictions, the results of both detection methods do not indicate an incidence of *Trichinella* sp. in the Swiss domestic pig population. Owing to its sensitivity, specificity and efficiency of application, the ELISA test used has proved suitable for large scale testing of slaughtered pigs. By means of the digestion method *Trichinella* infestation were detected in 1.3% of the tested foxes. The fox isolate was maintained in vivo at the laboratory level. Subsequent RAPD fingerprinting analyses provided the evidence of *T. britovi* as infecting agent.

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Functional micromorphology of the Ostrich feather louse (*Struthiolipeurus struthionis*)

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Ostrich feather lice are economically important ectoparasites to the ostrich industry. As chewing lice they live and feed on the feathers thus spoiling the marketability of the plumes.

The lice were collected from infested feathers and fixed in 70% ethanol or Bouin's solution. After ultrasonic cleaning, they were dehydrated through graded alcohols, critical point dried, sputtercoated with carbon and gold, and viewed in a Leica Stereoscan 420 scanning electron microscope (SEM).

The SEM studies revealed three organs functionally adapted to holding onto and chewing the ostrich feather. The two tarsal claws of each leg grasp the barboles of the feather against three large stalked bulbous setae which are longitudinally ridged. The barboles may be further locked longitudinally behind the mandibles, which also have medial notches to hold and bite off sections of feather. Ventrally the left clypeus is flap-like and projects over the antero-medial groove of the head in which barboles are securely held. The extendable membranes of the haustellum are also housed in this groove. Sensory organs and setae were found on the antennae, while the maxillary palpi have only sensory setae.

These morphological specializations along with the bristle-like abdominal setae, enable these lice to live successfully on the ostrich feather.



Management of tick resistance : The use of an acarine growth regulator

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Resistance of ticks to various acaricides constitutes worldwide an increasing problem and there is a need for new molecules with different modes of action and new concepts of tick control. Fluzuron (Acatak®) is a novel systemic acarine growth regulator with long lasting activity, specially suited for the control of ticks of the genus *Boophilus*. Fluzuron interrupts the tick life cycle at different stages by interfering with the chitin synthesis process. As the substance is devoid of cross-resistance, its use is particularly indicated in cases where ticks loose their susceptibility to known acaricides. In South Africa, the substance was applied as a pour-on on cattle artificially infested with *B. decoloratus* at doses of 3mg/kg, 2 mg/kg and 1 mg/kg. Control of 98 % or higher was achieved between days 28 and 40 after application at the doses of 3 and 2 mg/kg. At 1 mg/kg, control varied between 60 and 100 % during the same period. Plasma levels correlated directly to the applied dose and peaked at 0.09 ppm, 0.06 ppm and 0.03 ppm respectively after approximately 15 days. Statistical analysis of the results showed a EC plasma level of 0.0242 ppm for 90% control, 0.0295 ppm for 95% control and 0.0403 ppm for 99% control. While the dose of 1mg/kg is not suitable for long term control, a single administration of 2mg/kg and 3 mg/kg will provide plasma concentrations higher than or equal to the EC 95 and EC 99 levels for a period of 42 days, starting from the fifth day after application. Field trials are now being run by SABS to confirm these findings.

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The micromorphology of the African blue louse (*Linognathus africanus*)

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The African blue lice are economically important ectoparasites of goats and sheep. As bloodsucking lice they not only cause much irritation, but heavy infestations may cause severe anemia particularly in young animals.

The lice were collected from infested goats and fixed in 70% ethanol or Bouin's solution. After ultrasonic cleaning, they were dehydrated through graded ethanols, critical point dried, sputter coated with carbon and gold, and viewed in a Leica Stereoscan 420 scanning electron microscope (SEM).

The SEM studies revealed further specialization of the legs as grasping organs. Each ends in a stout tarsal claw which closes around the hairs clasping them firmly against pretarsal sclerites. The claws increase in size from the first pair with a small pretarsal sclerite, to the robust claw of the third pair of legs which closes against large pretarsal sclerites consisting of three pads which are ridged to improve the grip on the hair. A small stellate structure with a suggested sensory function was seen on the median base of the third claw.

The external morphology of the exoskeleton is scale-like in structure, with points directed posteriorly. The large thoracic and smaller abdominal spiracles are lined with circular rims. The anterior haustellum has a dorsal opening from the hypopharynx and a ventral opening of the salivarium. The distal segment of the antenna ends in sensory setae, while sensory 'pore organs' occur on the lateral surfaces of the two distal segments.

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It has been shown that biological control of parasitic nematodes of domestic animals can be achieved by feeding host animals spores of the nematode-killing fungus *Duddingtonia flagrans*. In the host faeces, *D. flagrans* develop small sticky hyphal nets (traps) that may catch parasitic nematode larvae and kill them. In experiments on corn meal agar *D. flagrans* has a growth rate between 15 and 60 mm/week at temperatures between 20 and 30°C. The presence of *Ostertagia ostertagi* and *Cooperia oncophora* larvae induces the fungus to produce increasing numbers of nets as a result of increasing number of larvae up to a concentration of approximately 20 larvae/cm². The rate of net-formation in *D. flagrans* has an optimum at 30°C, producing 700-800 nets/cm²/2 days, when induced by 20 parasitic nematode larvae/cm² on agar. Each net is a potential trap for at least one nematode. Approaching 10 and 35°C the ability to produce nets is gradually reduced. On agar, at 10, 20 and 30°C *D. flagrans* loses its net inducibility after 2 to 3 weeks. During the ageing process, increasing numbers of spores are produced up to a certain limit. The time for reaching maximum spore concentration coincided with the time for loss of induction potential. Abiotic factors such as low oxygen tension, dry conditions and direct light reduces the net formation.

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Toxoplasma gondii infection in the mountain hare (*Lepus timidus*)

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Pathological lesions and dissemination of parasites in the mountain hare (*Lepus timidus*) were studied after experimental infection with *Toxoplasma gondii* oocysts. Tissue samples were subjected to conventional histopathology and to immunohistochemistry using a specific anti-*T. gondii* antibody one week post inoculation. Also, serological assays for specific immunoglobulin (Ig) G and IgM production as well as a lymphocyte stimulation test (LST) for demonstration of *T. gondii* induced mitotic activity were used in search for early immune reactions of importance.

Histologically the inoculated animals showed extensive necrotic areas in the small intestine, mesenteric lymph nodes and liver, and less prominent focal necroses in a variety of other tissues. Immunohistochemically, *T. gondii* parasites were identified in all organs examined and they were numerous in connection with tissue lesions. Low titres of IgG and IgM indicated an early humoral response. However, lymphocyte cultures from hares showed no proliferative response to *T. gondii* antigen.

The outcome of primary *T. gondii* infection in the mountain hare was analogous to previous field observations in this species, and further emphasizes an unusually high susceptibility to fatal toxoplasmosis. The lack of an early cellular response to *T. gondii* might contribute to the severity of disease.

The impact of nematode parasites on milk production in dairy cattle: a review.

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An exhaustive literature search identified publications describing the impact of anthelmintic treatment on milk production in dairy cattle. From more than 60 experiments described in over 50 publications, trial designs were divided into four general categories, based on number of herds within a trial (one vs many) and duration of observation period (full lactation vs partial lactation). The treatment groups were untreated control and (one or more) dewormed groups. Anthelmintics investigated included members of the imidazothiazole, benzimidazole and macrocyclic lactone endectocide groups. Treatments were generally administered between drying-off and the first half of lactation. The number of experiments in which the medicated (or uninfected) group had numerically greater production was compared to the number of experiments in which the control (or infected) group had greater production, using the sign test. For milk production, 49 of 63 experiments (78%) had a positive response ($p < 0.001$). In each of the design categories, a majority of the studies had positive responses. Milkfat yield (weight of fat) was greater for medicated cows than controls in 23 of 31 experiments in which that variable was studied ($p = 0.012$). Fat percent, protein yield and percent and solids-not-fat percent were also greater for medicated cows than controls in a majority of the studies, but because only a few experiments examined these variables, statistical significance could not be demonstrated. The consistent response in favor of treated groups indicates that infection with nematode parasites can result in production losses in dairy cattle.

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FAO GIS data resources for animal disease control in Africa

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FAO information resources on agriculture and development are now routinely available as digital data that can be accessed via the internet or distributed on computer compatible media suitable for use in GIS animal disease control models. The FAO/NASA satellite image archive, available on CDROM for Africa with associated data analysis software (IDA, Addapix), includes normalized difference vegetation index (NDVI) data calculated for 1982-1991 from the AVHRR of the NOAA satellite series. More recent decadal (10-day interval) data from a rooftop receiver at FAO is accessible via the internet for NDVI and cold cloud duration estimates of rainfall. It is in current use for famine early warning, desertification, locust forecasts and other applications. The FAOCLIM worldwide climate database is available on CDROM. FAO Crop production system zone software (CVIEW) is designed to view, select and export over 500 environmental and crop production data variables on 1220 agroecologic units in the IGAD sub-region of East Africa to GIS software packages for specific applications. World agricultural statistics can be accessed via the internet or CDROM for the Global Information and Early Warning System (GIEWS) and AGRIS databases. FAO soil data are available on CDROM worldwide (1:5 million scale) and at 1:1 million for East Africa. An FAO central resource GIS unit provides higher level UNIX ARC/INFO software, hardware, data archives and training to support production of spatial databases and GIS applications using PC-based commercial GIS software (eg. Atlas, ArcView 3, Imagine, IDRISI, MapInfo and PCI). GIS initiatives in the FAO Animal Production and Health Division will be outlined, including efforts to develop comprehensive control models for trypanosomosis, helminths, rinderpest and foot and mouth disease.

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Cyclin dependent kinases (CDK's) are important for the orderly progression of the cell cycle. Some CDK related genes have been described in the plasmodia species but their role in parasite growth and differentiation is not known. To date three CDK related genes have been characterised in *P.falciparum* using PCR with specific primers. In trypanosomatids six different CDK-related genes have been isolated. The aim of this study is identify additional CDK-related genes of *P.falciparum* by using degenerate oligonucleotide primers and to determine whether their expression is stage specific.

The erythrocytic stage of *P.falciparum* was cultured *in vitro*. Total RNA was isolated from the ring and trophozoite stages of the parasite using the guanidinium isothiocyanate/phenol method. mRNA was reverse transcribed using both degenerate primers and random hexanucleotides. The resultant cDNA was amplified by PCR with degenerate primers. The primers were designed using the universally conserved amino acid sequences in the catalytic site of CDK's. In order to limit the degeneracy of the primers the codon usage of *P. falciparum* was taken into consideration. PCR products were analyzed by agarose electrophoresis. Purified products were blunt ended and subcloned into a vector. Sequence data were obtained using Sequenase.

RT-PCR yielded a major product of 550 base pairs and minor products of 150, 250, 800 and 1200 base pairs. Preliminary data indicate that these products are only expressed during the trophozoite stage of the parasite. Partial sequence data indicate that the 550 base pair band contains more than one CDK related sequence.

RT-PCR is useful in the identification of new genes from scarce biological material and is sensitive enough to obtain information on stage specific gene expression. The use of degenerate primers expands the application of PCR by identifying related and homologous gene sequences.

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Differentiation of eggs and larvae from gastrointestinal nematodes by PCR

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Infections with gastrointestinal nematodes are of major economic importance for cattle. It is not possible (except for *Nematodirus*) to microscopically differentiate eggs of different trichostrongyle genera. Ribosomal DNA and their spacer regions proved to be useful for species/genus differentiation.

The second internal transcribed spacers (ITS-2) of the ribosomal DNA of *Ostertagia ostertagi*, *Cooperia oncophora*, *C. punctata*, *Trichostrongylus axei*, *Haemonchus contortus* and *Nematodirus helvetianus* were amplified by PCR using ITS-2 specific primers. After agarose gel electrophoresis bands were isolated, DNA extracted, cloned into the plasmid vector pCR II and sequenced.

Specific primers were designed and used for the direct selective amplification of genomic DNA from eggs or larvae recovered from faecal samples. Quantitative analysis of the amplicate allowed to estimate the relative population size of the different gastrointestinal nematodes in the original sample. (The work was supported by a grant from the German Research Council, DFG grant No SCHN 267/8-1 u. 2).

Adaptability of *Dirofilaria immitis* and its microfilariae to cat

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Four mongrel cats were experimentally infected with L3 100-123 of *D. immitis*, canine heartworm, and euthanized on day 243-245 of infection. The present study was conducted for discussing an adaptability of this parasite species to the cat. The infection was monitored with microfilariae counts, adult worm counts, ELISA with PBS-extracted antigen of *D. immitis* and histopathological examination. Throughout the infection, only cat No.1 became microfilaremic on day 201 of infection with normal microfilarial periodicity as compared with that of microfilaremic dogs. In four cats, the anti-*D. immitis* ELISA titers were increased gradually after infection, but cat Nos.3 and 4, the titers were decreased after around day 130 and day 60 of infection, respectively while cat Nos.1 and 2 showed an increased titer curve, although cat No.2 never appear a microfilaremia until the end of the experiment. Adult worms were recovered from cat No.1 (3 males and 7 females, and 2 dead worms in pulmonary arteries (PA), 11.4% of recovery rate) and cat No.2 (1 male and 1 dead worm in PA). These results are indicative of enough adaptability of *D. immitis* to the cat.

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Possible effects of a primary *Schistosoma japonicum* infection on a superimposed *Ascaris suum* infection in pigsA.B. Helwig¹, H.O. Bøgh¹, P. Nansen¹, N.Ø. Christensen² and M.V. Johansen²
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The study was designed to examine the impact of a primary *Schistosoma japonicum* infection on the establishment and location of a superimposed *Ascaris suum* infection. A *S. japonicum* infection causes hepatic lesions as a result of the hosts immune reaction to trapped eggs. Therefore, migrating *A. suum* larvae might be arrested in its development during the liver passage in *S. japonicum* infected pigs.

The study comprised of two experiments each containing two groups: one group was primary infected with 1,000 *S. japonicum* cercariae and challenge infected with 1,000 *A. suum* eggs and the other group was infected with 1,000 *A. suum* eggs only. The *A. suum* challenge infections took place 11 or 16 weeks after the primary *S. japonicum* infection in experiment 1 and 2, respectively. All pigs were slaughtered 10 days after the *A. suum* challenge infections. The recovery of *A. suum* worms in the liver, lungs and small intestine which was divided into four equal sections was estimated. Furthermore, the mean ($\pm$ sd) number of *S. japonicum* eggs per gram (epg) liver tissue was obtained in order to check for successful primary infection.

In the first experiment the mean ($\pm$ sd) *S. japonicum* epg in the livers was 100 ($\pm$ 100) and moderate fibrotic lesions were observed. The mean recovery of *A. suum* worms was 604 ($\pm$ 205.5) for the *S. japonicum* infected group and 577 ($\pm$ 211.4) for the control group. In the second experiment the mean *S. japonicum* epg in the livers was 600 ($\pm$ 482.0) and severe fibrotic changes was observed. The recovery of *A. suum* worms was 449 ($\pm$ 110.1) and 402 ($\pm$ 125.7) for the *S. japonicum* infected and control groups, respectively. No significant difference in the recovery of *A. suum* worms was obtained between the groups in each experiment. The relative location of *A. suum* worms in the liver, lungs and sections of the small intestine was similar in the two groups in each experiment. Therefore, we conclude that a primary *S. japonicum* infection does not have an effect on the establishment and distribution of a challenge infection with *A. suum*.

Efficacy of doramectin pour-on in the treatment of lice and mange infestations in cattle

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Fourteen studies were conducted in Germany, France, Belgium, Ireland and Romania to evaluate the therapeutic efficacy of doramectin pour-on at a dosage of 500 µg/kg, administered topically to cattle harbouring naturally acquired infestations of either lice or mange. In each of the seven lice studies, between 13 and 22 cattle infected with one or more species of lice were randomly allocated to either a negative-control group or a doramectin-treated group. Lice counts were performed per species on each animal on a weekly basis for five weeks after treatment. Biting lice (*Damalinia bovis*) and sucking lice (*Haematopinus eurysternus*, *Linognathus vituli* and *Solenopotes capillatus*) were encountered in a minimum of two studies for each species. Across all studies, a single application of doramectin was 100% efficacious against all four lice species.

In each of the seven mange studies, a minimum of 14 cattle with confirmed active mite infestations were randomly allocated to either a doramectin-treated group or a negative-control group on the basis of their pretreatment mite counts. Following treatment, skin scrapings were taken from each animal at weekly intervals for five weeks and the mite population in each sample was enumerated. Single infestations of *Chorioptes bovis*, *Psoroptes bovis* and *Sarcoptes scabiei* were encountered in two, three and two studies respectively. In all studies, resolution of lesions on all treated animals was rapid and mite burdens were dramatically reduced by day 14. In five studies, doramectin efficacy was 100% and in the other two studies, efficacy was greater than 99%.

In summary, a single application of doramectin pour-on was highly efficacious against all species of the lice and all species of mange in cattle.

The life cycles of some oxyurid nematodes of the giant plated lizard, *Gerrhosaurus validus validus*

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Gerrhosaurus validus validus (Cordylidae: Gerrhosaurinae), is a large omnivorous lizard that frequents rocky outcrops in the north-eastern parts of southern Africa. Herbivorous lizards are often infected with oxyurid nematodes which all belong to the Family Pharyngodonidae. The nematodes have a direct life cycle and sometimes occur in large numbers in their hosts. The dearth of data regarding the nematodes of reptiles in general and their life cycles in particular prompted this study.

One adult male and one adult female plated lizards were trapped in the Timbavati Private Game Reserve. At autopsy, all the nematodes were collected and preserved in 70% alcohol, and samples of the faeces taken. The faeces were placed in a double container, the outer one of which was half-filled with water and kept at room temperature ($\pm 22^\circ\text{C}$).

Both lizards harboured about 1 500 nematodes and the male:female ratio was 1:10. The male lizard harboured only adult nematodes while both adult and immature stages were present in the female. Two kind of oxyurid eggs were seen in the faeces: larger ones, averaging $133.1 \times 73 \mu\text{m}$ and smaller ones, averaging $104.9 \times 64.5 \mu\text{m}$. Opercula were lacking in the larger eggs but were distinctly visible in the smaller ones. On Day 2 post collection both types of eggs contained morulae with only a few cells. A recognisable larva was first seen on Day 8 and 24 hours later the tail and the intestine, including the complete oesophagus with corpus, isthmus and bulb, were fully developed. Larvae expressed from the eggs were surrounded by the cuticula of the previous developmental stage. After Day 9 no further development of the larva in the egg could be observed.

We conclude that the first and second larval stages develop inside the egg and that the second stage larva, which is the infective stage, goes into a resting phase after Day 9. It remains in this phase until the egg is ingested by the next potential or suitable host.

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Topically applied insecticides of sheep - what hinders and what helps chemical dispersion

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The use of insecticides, particularly those of the synthetic pyrethroid (SP) chemical class, applied immediately after shearing (off-shears) as a single or multiple stripe along the sheep's back, is extensive. The efficacy of this mode of application relies on dispersion of SP concentrations that are sufficiently high to kill parasites, notably the sheep biting louse *Damalinia ovis*, which can reside in areas of infestation remote from the application site. Soon after shearing there is a "surge" in wool grease production but this returns to pre-shearing levels within approximately 10 days. Initially the grease is largely unoxidised and it is during this 10-20 day period that significant SP dispersion occurs. The grease progressively oxidises with concomitant reduction in SP dispersion. The oxidised grease now "entraps" the SP with the growing wool removing the insecticide from the skin surface, the site of lice predilection. Poor kill rates may be due to the lack of SP availability at the lice feeding site and/or lice not absorbing SP from the aged grease. Furthermore, decreasing availability may contribute to generation of SP resistance.

Greater use of non-oxidised wool grease components, considered to facilitate SP diffusion, should be made. Sterol and wax esters of wool grease remain largely unoxidised and when these compounds are used as a "carrier" increased dispersion of SP, over a longer period than with conventional formulations, occurred. Greater quantities of SP appeared to exchange with freshly secreted grease, increasing exposure, and lousicidal effect, to the skin feeding lice.

Intestinal Parasites in Working Equids in México

Part 1. The effect of 2 deworming treatments per year

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One of the important health problems in working equids (donkeys, mules and horses) in Mexico and other developing countries is intestinal parasitosis. The animals are mostly owned by poor peasants who are unaware of the importance of preventive medicine for their animals and who may not be able to pay for deworming medicines. The International Donkey Protection Trust started a project in Mexico, some 14 years ago, which mainly consisted at the time in periodic deworming programmes for donkeys. Later the International League for the Protection of Horses joined and the National University of Mexico has now integrated the project into their extension activities. Deworming medicines are expensive and it would be impossible to treat the animals as often as is done in riding establishments, race tracks etc. The present long term study is under way in order to find a less expensive deworming programme for working animals that would control their parasitic load, and to establish whether there are differences of parasitic loads between species. The study is carried out in a geographical area divided into a rainy and a dry season. The preliminary results of 372 equids studied during the rainy season are presented. 214 had never be dewormed and 158 had received 2 treatments during the past year one with ivermectine and one with oxybendazole. The group was divided in horses (112), donkeys (222) and mules (38). Rectal samples were taken and examined by the techniques of McMaster and flotation. The preliminary results indicate a highly significant ($P < 0.001$) difference between the twice treated and the untreated animals. However the egg count in the treated group remained too high to be satisfactory. The main parasites are: *Strongylus* sp., *Parascaris equorum*, *Strongyloides westeri*, *Oxyuris equi*, *Gasterophilus* sp. Egg counts are lower in donkeys than in horses, and lowest in mules. Work is continuing to find a strategic and sustainable programme considering the particular conditions in México.



Evidence for a parasite-mediated block of abomasal acid secretion in sheep infected with *Ostertagia leptospicularis*

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The ability of the fundic mucosa to acidify abomasal contents was studied in 6 sheep experimentally infected with 80'000 third-stage-larvae of *Ostertagia leptospicularis* and in 2 sheep into which adult *O. leptospicularis* had been transplanted. The acidity of the abomasal contents was continuously recorded using a pH probe which was introduced through a rumen fistula and was permanently located in the abomasum. In the sheep infected with larvae the abomasal acidity decreased markedly; pH levels > 4 were recorded after days 15 to 18 post infection (p.i.) and remained high until anthelmintic treatment. At days 19 and 20 p.i. when the mean pH ranged between 5 and 6, each sheep was administered 80 µg/kg histamine s.c. and 8 µg/kg carbachol respectively at different occasions. Both drugs caused an acidification of the abomasal contents reaching a mean of pH 3.4 within 1 hour after drug administration. The effect of carbachol could be completely blocked with the H2 antagonist ranitidine, suggesting that the final target of both drugs was the parietal cell H2 receptor. One to 4 days after anthelmintic treatment with ivermectin and fenbendazole the abomasal acidity returned to normal levels (pH 2 - 3) in all sheep.

In the 2 sheep transplanted with adult *O. leptospicularis* the abomasal pH increased within 10 hours reaching values of 4 and 6 respectively and remained elevated in the sheep receiving the higher adult population (approx. 16000).

The results indicate that the mucosa of the infected sheep harboured a population of functional parietal cells. Despite high serum gastrin levels the acid producing capacity of the parietal cells was directly or indirectly blocked by an unknown factor mediated by the parasites. The onset of severe reduction of acid secretion was closely related with the onset of parasite egg production.

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Genetic analysis of drug resistance in *Haemonchus contortus* using microsatellites

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Microsatellite markers are a useful tool to analyse parasitic nematode population structures and the effects of selection pressure. Microsatellites are tandemly repeated short simple sequences (1-6 base pairs), that are polymorphic due to the variability in the number of tandem repeats. We isolated and characterized 13 microsatellites of the sheep parasite *Haemonchus contortus*. The majority of these markers were polymorphic in one or more populations. This allowed for the easy discrimination of populations and individuals within a population. The effects of drug treatments on *H. contortus* populations were investigated by analysing microsatellite alleles from susceptible populations and from populations resistant to benzimidazoles and/or levamisole. Striking effects were identified even on populations that were only once treated with the drugs. These results indicate that few drug treatments can have extensive effects on the genome of a parasitic nematode.

Looking for AT repeats

3000 colonies 246 web

(CA)₁₅ - 0190 - 12 positives

(CA)₁₅ - 0190 -

do it get perfect repeats as in vertebrates

ATx3 < ATx4

fewer than vertebrates

GIS for medical applications in Africa : Getting started

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Geographic information systems technology provides new ways to use computers to create, archive and analyze traditional map data in the epidemiology of disease and to combine these with global environmental data sources derived from sensors on board earth observing satellites. A GIS is created by linking standard computer database 'attributes' to map features represented as layered point, line and polygon 'vector' data or to features represented as digital 'raster' image data pixels (picture elements). Using a new generation of commercially available digital geographic databases and hand-held global positioning systems (GPS) devices linked to a constellation of satellites, precise point locations and maps can be created for disease applications. Basic GIS and GPS concepts and requirements for construction of a GIS will be presented. Common software/hardware and data sources available for PC-based GIS will be described and suggestions provided on how to establish a PC GIS capability for investigation of disease at costs of less than \$25,000. Examples of how to obtain training and link medical GIS with larger national GIS programs such as those in Botswana or Cote d'Ivoire, will be presented.

Some populations with some markers are polymorphic for some markers but not all

variation

H. contortus has 6 chromosomes

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Epidemiology of *Anoplocephala perfoliata* infection in foals on a stud farm in south-western Sweden

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The egg output and humoral antibody response to scolex antigens of the equine tapeworm *Anoplocephala perfoliata* were monitored in naturally infected foals by an egg flotation/centrifugation method and an indirect enzyme-linked immunosorbent assay (ELISA). The study was performed on a stud farm in south-western Sweden between May 1994 and April 1995. Sequential blood and faecal samples were taken from 21 foals starting at about 4 weeks of age and then approximately every fourth week throughout the studied period. Results were expressed separately for 10 and 11 foals born before and after the end of April 1994 respectively. Increased levels of antibodies were noticed from mid September and onwards in both groups whereas tapeworm eggs were detected in the faeces of all foals about 3 months later. The antibody response was similar in both groups but it was more pronounced in foals born before April 1994. All foals were treated in March 1995 with an oral paste formulation of pyrantel pamoate at a dose rate of 38 mg/kg bodyweight. Most animals responded to the anthelmintic treatment and one month later, tapeworm eggs were only detected in one out of the 21 foals dosed. Thus, the treatment reduced the number of *A. perfoliata* egg positive horses by 95%. A concomitant decline in antibody levels was also observed. Western immunoblot analysis of sequential individual serum samples showed at least ten different scolex antigens in the molecular weight range 10-200 kDa were recognised. Banding intensities, especially of the 10 kDa, 35 kDa, 45



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The cystforming coccidian parasite *Neospora caninum* has emerged as a major cause of abortion in cattle in many countries. In an effort to gain better understanding of this parasite at the molecular level we have sequenced and analysed a large number of mRNAs transcribed from the *N. caninum* genome.

N. caninum (NC Liv. strain) tachyzoites were maintained in a vero cell culture. Total RNA was isolated from purified tachyzoites and further processed to isolate mRNA by oligo-dT cellulose chromatography. A cDNA library was constructed from *N. caninum* mRNA using the ZAP cDNA synthesis kit and cloned into the UNI ZAP XR bacteriophage vector. Well isolated recombinant phage plaques were picked and the inserts were amplified by PCR with primers complementary to the phagemid. Amplicons were purified by a Qiagen PCR purification kit and run on a 1 % agarose gel with a LambdaDNA/HindIII marker to estimate concentration and purity. The amplicons were sequenced by cycle sequencing and run on an ABI automated DNA sequencer (Supamac, Sydaey, Australia). The sequences obtained were edited and used to search DNA and protein (in all 6 reading frames) sequence databases for significant identities.

A total of 707 random plaques were picked and of these 528 gave DNA templates for sequence analysis with an average insert size of 1100bp. Database searches showed approximately 65 % of the sequences had significant matches. Of these 31 % were homologous to previously identified *Toxoplasma gondii* ESTs. The remaining ESTs probably represent unique *N. caninum* sequences.

In conclusion, EST sequencing has rapidly provided high quality sequence data from a large number of independent genetic loci found in the *N. caninum* genome. Several of these ESTs are homologous to genes of other protozoan parasites that have been previously identified as candidates for the development of genetically engineered vaccines and for therapeutic drug development.

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A total of 857 fecal samples from cattle was examined at several dip tank areas in the Northern part of Malawi at the end of the dry as well as of the rainy season 1992/93. Egg excretion rate of the two milk transmitted helminths *Toxocara vitellorum* and *Strongyloides papillosus* in calves in their first months of age reached highest levels of 22% and 68% respectively. The occurrence of ascarids was restricted to particular herds and did not exceed 22%. *S. papillosus*, in general, was widespread over all herds, however, the intensity of egg output was low. Control programmes of strategic chemoprophylaxis in calves up to three weeks should be established only in risk herds. The prevalence of gastro-intestinal strongylids increased from the second months of age onwards more rapidly after the rainy season, reaching a peak plateau of 90% to 100% at one year of age. However, the intensity of egg excretion was low. Egg excretion of *Fasciola gigantica* and *Paramphistomum* spp. started between 6 and 8 months of age. The prevalence of liver flukes on the basis of egg output did not exceed 10 - 12% during the first two years of life and reached maximum values of about 53% only in older animals. Amphistome infections, in contrast, increased at a faster rate and maximum values of nearly 80% were reached more rapidly in all herds.

80% of blood smears taken from 739 animals were positive for intraerythrocytic stages of *Theileria* spp., and more than 25% of 4 weeks old calves were already infected. In several areas at an altitude of 1,600 to 1,700 m above sea level only a few animals were infected, whilst at the other dip tank areas of the Central Plains and the Lake Shore the prevalences reached up to 100%. In general, clinical signs were not observed in the calves examined. Therefore, it is assumed that most cases were *T. mutans* infections. The prevalences of *Babesia bigemina* and anaplasmas were low, ranging between 2% and 5%.

While the impact of various helminth infections in calves in the Mzuzu ADD was independent from seasonal influences, the prevalence of theileriosis did increase at the end of the rainy season, when the tick infestation rate of *Rhipicephalus appendiculatus* was at the peak levels. Higher infestation rates of babesiosis, in contrast, were exhibited during the dry season concurrent with the higher activity of adult *Boophilus* ticks.

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Fleas and associated skin diseases of dogs and cats are one of the commonest reasons for pet owners to visit veterinarians. Control methods are usually based on flea adulticide treatments for the animals plus physical and chemical control measures for immature stages in the surroundings.

Imidacloprid in a 10% w/v topical formulation is a new product which provides both removal of existing flea infestations, protection from re-infestation for a month and provides a larvicidal effect in the surroundings of the treated animals.

The adulticidal effect was tested on 12 flea infested dogs at commercial label rates and in 10 cats at the minimum recommended label rate. After a single treatment the dogs were reinfested with 150 fleas weekly for 7 weeks and the cats with 100 fleas weekly for four weeks. Fipronil as a 9.7% topical formulation at recommended label rates was used on five dogs as a positive control.

Flea counts were conducted 30 hours after artificial reinfestation in dogs and after 24 hours in cats. All flea eggs dropping from the cats over a two hour period were recorded 48 hours after the initial treatment and 72 hours after each reinfestation.

Results are tabulated below.

Percent reduction in flea or egg counts after treatment on day 0

Days	+1	+7	+14	+21	+28	+35	+42	+49
DOGS								
Imidacloprid	100%	100	100	99.1	99.6	99.3	98.1	88.1
Fipronil	84.6	100	100	100	99.5	98.5	95	72.2
Untreated group.	43 to 61 fleas per animal over the trial period							
CATS								
Imidacloprid								
Adult fleas	99.5	99.1	96.9	96.2	95.7			
Flea eggs	98.5	100	99.9	99.9	97.8			
Untreated group.	20 to 24 fleas per cat over trial period							
	169 to 243 eggs dropped per cat in 2 hours.							

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The first internal transcribed spacer (ITS-1) of the ribosomal DNA of 6 species of *Trichostrongylus* has been sequenced. The length of ITS-1 for the different species varied from 385 to 388 bases and the G+C content was approximately 42 %. The level of intraspecific variation was estimated for 3 species by comparing the ITS-1 sequence from different geographical isolates. No intraspecific variation was detected for *Trichostrongylus axei* and *Trichostrongylus colubriformis* and a low level (0.5 %) was found for *Trichostrongylus vitrinus*. In contrast, the level of interspecific variation between species ranged from 2.1 % to 5.7 %. The highest levels of sequence differences was detected between *Trichostrongylus tenuis*, the species which infects birds, and the 5 species which occur in mammals. Some of the nucleotide differences occurred at sites corresponding to recognition site for endonucleases. These results are compared with previous data obtained on the other spacer of the rDNA, ITS2, and indicate that ITS-1 may also be useful for taxonomic and phylogenetic studies.

The effect of corticosteroid treatment of *Trichostrongylus colubriformis*-infected sheep on the LD₅₀ of ivermectin in an *in vitro* larval development assay

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Previous results from larval development assays with *Ostertagia circumcincta* have shown that the LD₅₀s of ivermectin (IVM), thiabendazole (TBZ) and levamisole change with time after a single infection, rising to a peak at about 50 days post infection (DPI) and then returning to baseline values (Amarante *et al.* in press). Similar observations were made with TBZ and *Haemonchus contortus* in an egg hatch assay (Borgesteede and Courwenberg 1987). The aim of this experiment was to investigate if this same phenomenon was apparent with *Trichostrongylus colubriformis* and what effect corticosteroid treatment of the host sheep has on this change in LD₅₀.

Six field-reared lambs, about 3 months old, were effectively treated with an anthelmintic, housed and infected with 35000 infective larvae (L3s) of *T. colubriformis*. From 10 DPI, 3 lambs (Group 1) were treated once weekly with 0.5mg kg⁻¹ of dexamethasone trimethylacetate whilst the other 3 (Group 2) remained untreated. Eggs were collected weekly from 21 DPI to 112 DPI, mixed with a nutrient media, layered over agar gels in microtitre plates which contained varying concentrations of ivermectin and incubated for 7 days. The adjusted proportion of L3s and log concentration of IVM were fitted to a sigmoid curve (Slidewrite, v. 6, Advanced Graphics Software Inc.) to estimate the LD₅₀.

The LD₅₀s for Group 1 remained at about 0.05Fg ml⁻¹ until 35 DPI and then declined to about 0.01Fg ml⁻¹ on 42DPI at which level they remained for the duration of the experiment. The LD₅₀s for Group 2 were similar to Group 1 until 35 DPI. They continued to remain at this level until about 49 DPI after which they steadily climbed to a peak of 0.20Fg ml⁻¹ on 70 DPI and then steadily declined to about 0.05Fg ml⁻¹ on 84 DPI where they remained for the duration of the experiment.

These findings demonstrate that :

(1) there is a similar change in LD₅₀ with time for *T. colubriformis* as with other trichostrongyloid nematodes tested.

(2) following steroid treatment the rise and fall in LD₅₀ is not seen implying this change is due in some way to the immune response of the sheep or its absence is due to a direct effect of the steroids on the worms themselves.

Ingesting

The *in vitro* effects of acetylcholinesterase secreted by 2 nematode species, parasite of the small intestine, on the growth of HT29-D4 cell line.

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Acetylcholinesterase (AChE) is secreted by several nematode species, parasite of the digestive tract of domestic ruminants. The quantity of enzyme as well as the molecular forms secreted differed between species. The role of this enzyme for the worms remains undefined. Previous *in vitro* results have indicated that excretory /secretory (E/S) products of *Trichostrongylus colubriformis* and *Nematodirus battus* were responsible for changes in the growth of the epithelial cell lines HT29-D4 and HGT-1 (respectively from colic and gastric origin). A role of AChE secreted by those worm species has been suspected and the present study aimed at verifying this hypothesis.

The effects of purified AChE from both worm species on HT29-D4 cell line have been examined. Purification of the enzyme was performed by edrophonium affinity chromatography. The AChE was added to the cell medium culture at concentrations ranging from 0.1 to 14 Units /ml (U/ml) for *Trichostrongylus colubriformis* and 0.2 to 343 U/ml for *Nematodirus battus*. In addition, AChE from electric eel (Sigma) was tested (2 to 2000 U /ml) on the same cell line to assess the specificity of the effect. The growth of HT29-D4 cells was measured by MTT method and tritiated thymidine incorporation. A mitogenic effect was detected with AChE from both nematode species in a range of concentrations between 0.2 to 14 U/ml. On the other hand, an inhibition of cell growth was found with high concentrations of the enzyme from *N. battus* (343 U /ml) or electric eel (2000 U /ml). In addition, the mitogenic effect of the ES products from *Trichostrongylus colubriformis* disappeared after depletion in AChE.

These results suggest thus a role of AChE secreted by the parasitic nematodes in the epithelial regeneration of the gut.

Effects of excretory / secretory products of 6 nematode species, parasite of the digestive tract of ruminants, on the *in vitro* proliferation of 2 cell lines (HT29-D4 and HGT-1) of epithelial origin.

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Parasitism of the gastrointestinal tract of domestic ruminants with different nematode species is often associated with an increased regeneration of the digestive epithelium. A mitogenic effect of the excretory/secretory (E/S) products of *Trichostrongylus colubriformis* has previously been assessed *in vitro* on the cell line from epithelial origin HT29-D4. To examine the specificity of this effect, the E/S products of 5 other species have been incorporated in a range of concentrations to the culture medium of HT29-D4 cell line and the cell growth was measured using the MTT method. The effects of the substances, released by the 5 worm species and *T. colubriformis* were also assessed on the gastric cell line HGT-1. A mitogenic effect on the HT29-D4 cell line has been observed for the E/S products from 3 out of 4 species, parasite of the small intestine (*T. colubriformis*, *T. vitrinus* and *Cooperia curticei*). For the last intestinal species (*Nematodirus battus*), a reduction in the number of cells was on the contrary measured. Among the abomasal species examined, an increased proliferation was associated with the E/S products from *Teladorsagia circumcincta* whereas those from *Haemonchus contortus* have no effect. The E/S products of all 6 species gave a similar increase in proliferation of the HGT-1 cell line of gastric origin. These results suggest that the E/S products released in the environment by the parasitic nematodes may play a major role in the host-parasite relationship. The mitogenic effect previously observed *in vitro* with *T. colubriformis* did not appear specific nor of the nematode species neither of the cell line. In addition, the effects are not limited to the cell line corresponding to the anatomical location of the worms.

Characterisation of equine strongyles by ribosomal spacer DNA sequences : implications for diagnosis and systematics

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Infections of horses with parasitic nematodes of the Order Strongylida are of major veterinary importance. There are over 50 species belonging to two subfamilies, the Strongylinae ("large strongyles") and the Cyathostominae ("small strongyles" or "cyathostomes"). The identification of these parasites to the species level is particularly important for the diagnosis of infections and for studying their epidemiology and taxonomy. However, there are considerable limitations in identifying some stages (in particular eggs and larvae) to species using morphological features.

DNA technology provides useful alternatives for parasite identification. In particular, PCR-based methods have the sensitivity required to identify individual parasite stages from small amounts of material, and ribosomal rDNA can provide the genetic markers for the identification of parasites to species. We are currently employing direct PCR-based DNA sequencing to characterise the first and second internal transcribed spacer (ITS) of rDNA for a range of equine strongyles. So far, the ITS sequences of 17 species have been determined. Intraspecific sequence variability is low (< 1 %), while interspecific differences range from 1.5-50 %. The data demonstrate that each species can be identified unequivocally by its ITS sequence. The definition of genetic markers in ITS for the identification of equine strongyles to species provides the foundation for establishing PCR-based tools for diagnosis of infection and for studying the systematics and phylogeny of this group of parasites.

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Research in Veterinary Parasitology in Anglophone West Africa has suffered considerable setback in the last 10-15 years, primarily due to macro-economic and political factors. Many parasitic problems for which successful control programmes had been instituted have again become major concerns because of lack of sustaining surveillance and control measures. Research institutes have been badly depleted of trained and qualified manpower and equipment, and remaining staff have become frustrated due to lack of incentives. The universities have not fared much better either. Increased urbanization due to rural-urban drift has further complicated the picture in the area of human parasitic infections. Above all, the subregion has been bedeviled with social and political instability.

In assessing research needs, therefore, due cognizance must be given to these and other broad factors which constrain research in parasitology in the subregion. Some of these are:

- Macro-economic and social problems, political instability and fiscal collapse with, in some countries, massive devaluation of the national currencies;
- The decline and, presently, lack of funding both at the national and institutional levels, that have led to: i) breakdown of the infrastructure of institutions created during the pre- and post-independence era; ii) lack of maintenance of infrastructure and equipment as well as inadequate remuneration for staff, leading to iii) brain drain of human resources to better-endowed regions such as to Europe, U.S.A. and some parts of southern Africa;
- The decline in donor support, including the support provided specifically by donors and industry for research in parasitology and parasitic diseases.

Given this scenario, the need to restore even minimal capacity building not only in parasitology research but also in scientific research has become more urgent than ever before. The first step would appear to be to bring about restoration of infrastructure and provide fiscal encouragement.

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Serodiagnosis of echinococcosis in humans and animals

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Echinococcosis, either alveolar or cystic, is one of the most serious zoonotic helminthic diseases in humans and domestic animals. The former is caused by oral ingestion of eggs of *Echinococcus multilocularis*, whereas the latter by eggs of *E. granulosus*. In this paper, I would like to introduce my recent work on serodiagnosis of echinococcosis in humans and animals.

Alveolar echinococcosis by *E. multilocularis*: Em18-Western blot is highly reliable for differentiation of alveolar echinococcosis (AE) from cystic echinococcosis or cysticercosis. Almost all AE human cases so far examined except few with calcified lesions only and the wild vole, *Clethrionomys rufocanus bedfordiae* (the suitable intermediate host), naturally infected with this parasite showed clear antibody response against Em18. In contrast, the rat, *Rattus norvegicus*, is known to be resistant and unsuitable host for this parasite, in general. When rats were experimentally inoculated with protoscoleces of *E. multilocularis* intraperitoneally, they became infected with huge number of protoscoleces after six months at least. It was easy to prepare 30 - 40 ml packed protoscoleces from a single rat. However, when we could check antibody responses of *R. norvegicus* naturally infected with this parasite and *Taenia taeniaeformis*, they showed no antibody responses against *E. multilocularis* or *T. taeniaeformis* at all. Therefore, it is suggested that *R. norvegicus* may only be the intermediate host for *E. multilocularis* under some exceptional condition such as immunosuppression.

Cystic echinococcosis by *E. granulosus*. Antigen B appeared to be the most sensitive antigenic components of cyst fluid of *E. granulosus* but not to be species specific. Antibody responses against crude antigens of *E. multilocularis* protoscoleces (EmPS) appeared to be more sensitive and specific in AE and CE human sera except that antibody against Em18 is specific to AE. Serodiagnosis using EmPS is recommended for AE and CE.

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Trypanosoma brucei gambiense: Importance of CD4⁺ T cellN. Inoue¹, D. Narumi², M. Inoue¹, K. Hirumi¹, I. Igarashi¹, H. Nagasawa¹, A. Saito², N. Suzuki² and H. Hirumi¹

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In general, *Trypanosoma brucei rhodesiense* is more virulent to mice than *T. b. gambiense*. *Trypanosoma b. rhodesiense* IL2343 (IL2343), originally considered to be *T. b. gambiense*, causes fatal infection with high parasitemia in mice, killing the hosts within a month. While, *T. b. gambiense* IL3253 (IL3253) causes chronic infection with sporadic and low parasitemia in mice which can overcome the parasite. The objective of this study is to clarify the role of T cells in protective immunity against high (IL2343) and low (IL3253) virulent trypanosomes.

Anti-CD4 (ATCC TIB-207 GK1.5) and anti-CD8 (ATCC TIB-105 53-6.72) monoclonal antibodies were used to deplete T cell subsets in BALB/c mice. Experimental groups were consist of 4 groups (i.e. normal, CD4⁺ T cell-depleted, CD8⁺ T cell-depleted and both CD4⁺ and CD8⁺ T cells-depleted, five mice/group). Mice were infected (i.p.) with 50 bloodstream forms (BSFs) of IL2343 or IL3253. Every day, the number of parasites in the blood was counted by using a hemocytometer.

In IL3253-infected groups, all the mice survived the infection up to the end of the experiment (30 days after infection). Parasitemia in CD4⁺ T cell- and both T cell subsets-depleted mice persisted throughout the experiment at a range between 10⁴ to 10⁶ BSFs/ml. This pattern of parasitemia was similar to that of athymic nude mice. Control and CD8⁺ T cell-depleted mice showed sporadic and low parasitemia. While, all the mice infected with IL2343 died within eleven days with high parasitemia.

These results indicated that CD4⁺ T cells play an important role in controlling the parasitemia during low virulent *T. b. gambiense* infection in mice.

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Cysticercosis: Laboratory animal model and serodiagnosis in humans and pigs

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Cysticercosis is one of the most serious zoonotic helminthic diseases in humans and domestic animals. *Taenia solium* cysticercosis is most important for public health and economic problem. As the intermediate hosts for human *Taenia* spp. such as *T. saginata* and *T. solium* or so-called Asian *Taenia*, are cattle and pigs, respectively, it is not easy to do experimental work. We have established severe combined immunodeficiency (scid) mice as laboratory animal models for the intermediate host of human *Taenia*. When in vitro hatched oncospheres of *T. saginata*, *T. solium* or Asian *Taenia* were inoculated into scid female mice subcutaneously or intraperitoneally, they developed into fully mature cysticerci. Therefore, now we can compare the cyst fluid antigens of various taeniid species developed in the homogeneous mouse host instead of different domestic animals.

Immunoblot analysis has been carried out for differential serodiagnosis of cysticercosis in humans and pigs. Almost all sera of cysticercosis from humans and pigs have shown very similar specific antibody responses against partially purified antigens of *T. solium* cysticerci. There is no cross reaction with alveolar or cystic echinococcosis at all. It is expected that serodiagnosis of cysticercosis bovis in cattle due to *T. saginata* may be feasible based on the similar strategy.



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The performance of groups of ewes, treated prior to lambing and their twin lambs, that were either untreated or treated with fenbendazole, ivermectin, levamisole or with both fenbendazole and levamisole, was followed over three successive grazing seasons. The animals were set stocked (10 ewes and twin lambs/hectare) on pastures naturally contaminated with populations of benzimidazole resistant *T. circumcincta* and drug susceptible *N. battus* and *T. vitrinus*.

There was no clinical evidence of teladorsagiosis in any of the lambs during the study and the average maximum difference in lamb weight gain between the fenbendazole treated and the best performing group was 3.1 kg. No differences were seen in the immunogenicity and pathogenicity of the field isolate of *T. circumcincta* compared to benzimidazole susceptible and benzimidazole and ivermectin resistant isolates.

Both ivermectin and the fenbendazole/levamisole combination treatment remained highly effective throughout but there was some evidence of increasing resistance against levamisole by the end of the study.

Some increase in efficacy of fenbendazole against *T. circumcincta* was achieved through feed withdrawal prior to treatment, or by dividing the dose or by co-administering piperonyl butoxide. The results from this study suggest that anthelmintics against which resistance has already been selected still offer some benefit in temperate climate production systems where resistance involves less pathogenic species with a relatively low biotic potential such as *Teladorsagia*.

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PCR-based methods for identification of *Toxocara* spp. and *Toxascaris leonina*

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Genomic DNA was extracted from ascaridoid nematodes collected from dogs, foxes and cats. A region spanning the second internal transcribed spacer (ITS-2) of the ribosomal DNA of each sample was amplified by PCR. Representative ITS-2 products for each nematode species (*Toxocara canis*, *Toxocara cati* and *Toxascaris leonina*) were sequenced. Restriction sites were identified for use as genetic markers in a PCR-linked RFLP assay. The three species could be differentiated from each other and from other ascaridoids that may be found in human tissues by use of two endonucleases, *HinfI* and *RsaI*. Primers were designed to unique regions of the ITS-2 sequences of the three species for use in diagnostic PCR procedures and primer sets evaluated against panels of homologous and heterologous DNA samples. Results suggest that both methods are good candidates for further development for the detection and/or identification of ascaridoid larvae in human tissues.

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Comparative clinical trials with a new compound, imidacloprid, and lufenuron against fleas (*Ctenocephalides felis*) in cats

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Controlled pen studies were conducted to investigate i) the efficacy of a 10 % spot-on formulation of imidacloprid against a resident flea population; ii) the duration of activity of a single dose; and iii) the prophylactic potential of a treatment programme.

In the first study, 20 cats were allocated to 4 matched groups. Cats in two groups were treated once with imidacloprid (10mg/kg). All were given 60 fleas one day before, and at weekly intervals after treatment. Surviving fleas were counted 24 and 48h after treatment and subsequent infections. Overall efficacy 24h after treatment was 99.8 %. Efficacy > 95 % persisted 3 - 4 wks when fleas were counted 24h after challenge, and 4 - 5 wks when fleas were counted after 48h.

The second study simulated a home environment by establishing a self-replicating flea population in each of three similar pens. One pen housed cats treated with imidacloprid; another cats receiving lufenuron. Treatments were given as per label instructions every 28 days. In addition to the naturally acquired challenge, each cat was given 5 extra fleas every week for the last 8 weeks of the study to mimic the roaming cat recruiting fleas outside the home. Fleas had to be removed from control cats 18 times to maintain burdens within acceptable welfare limits. In contrast, no fleas were found on any imidacloprid-treated cat during the 16 week study. Flea burdens of lufenuron-treated cats were reduced by 86.8 % after 6 wks but 3 welfare combings were required thereafter.

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Eradication of mange mite using one or two treatments of doramectin in swine herds with natural infestations of the disease

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Attempts to eradicate *Sarcoptes scabiei*, using intramuscular injections of 300 µg of doramectin (Dectomax[®], Pfizer, NY, USA) per kg of body weight, were made in 2 piglet-producing herds. In herd A, all animals were injected twice, while the animals were injected only once in herd B. No hygienic or other precautions were made in the herds. However, replacement gilts that were purchased after the acaricidal treatment of the herd were treated twice with doramectin in a quarantine before being introduced into the herd.

Prior to the injections with doramectin, the diagnosis was made by skin scrapings, and live *Sarcoptes scabiei* were isolated from both herds. Skin lesions similar to those caused by mange were defined on a scale of 0 to 3. Within this scale, pigs at the weight of 25 kg showed lesions corresponding to 1.3 ± 0.8 (A) and 1.0 ± 0.5 (B). The corresponding rubbing index values (15-minute period) were 0.95 (A) and 0.20 (B). Pigs at the weight of 25 kg expressed serum antibodies to *Sarcoptes scabiei* corresponding to optical densities of 0.16 ± 0.12 (A) and 0.11 ± 0.08 (B).

At intervals of 4 months, the herds were clinically examined for 20 (A) or 16 (B) months after treatments. Clinical signs of *Sarcoptes scabiei* were not observed, nor were mites demonstrated on any of these occasions. The mean of the skin lesions among 25-kg pigs had already dropped after 4 months. The mean value of the skin lesions were 0.1 ± 0.1 for both herds and the rubbing indexes were 0.13 ± 0.05 (A) and 0.14 ± 0.10 (B) during the period studied. Similarly, the levels of antibodies to *S. scabiei* in pigs at the weight of 25 kg rapidly decreased to 0.06 ± 0.02 (A) and 0.05 ± 0.03 (B).

Taken together, these results indicate that doramectin was efficient in eradicating sarcoptic mange. The drug appears to have the potential to eradicate the mange using only one treatment. However, if this is to be achieved, precautions aimed at properly administering the drug to all animals must be taken.

Tick infestation and transmission of *Borrelia burgdorferi sensu lato* at different density of steers on dry grassland; Preliminary results

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The purpose of the investigation was to study the infestation of *Ixodes ricinus* on cattle and transmission of the tickborne disease *Borrelia burgdorferi* at three different grazing intensities (7, 14 and 21 pr. 10ha) over a period of years. On inspection once each month peaks in number of ticks were found in June and September. The number of feeding ticks was high in 1993 (3.7 pr. steer (June/July)) and low in 1994 and 1995 (0.24 and 1.22 pr. steer (June/July)) showing correlation to the water balance for the areas (Regression analysis for water deficit and number of ticks pr. steer in July: $R^2 = 0.97$). The relative number of ticks pr. steer were 1.7 times higher (std: 0.37) at 0.7 compared to 2.1 steers pr. ha all years, while the calculated total number of ticks on the herd of 7 steers was 0.57 (std: 0.12) of the number of herd of 21 (June/July numbers).

Maximum transmission of *B. burgdorferi* occurred in 1993 (62 % sero-conversion) the following years the rate dropped to 48 % and 22 %. Sero-conversion occurred primarily in July and November, suggesting two peaks in numbers of infected ticks.

We believe that the low number of ticks in June 1994 was caused by an relative earlier questing period for nymphs and adults and that the steers avoided infestation due to difference in timing in relation to spring peak. The earlier activity did however not reduce infestation with infected ticks as the number of sero-conversions in June and July 1993 and 1994 were the same. The average sero-conversion at low density of steers was 32.2 % (Std: 5.4%) while the two other areas had a greater rate of sero-conversion (48.3 and 41.4 %; Std: 15.7 and 23.6 %). As the sero-conversion was greater at high density of steers although less ticks were counted pr. steer we conclude that grazing may increase the infection rate in ticks.

We propose that the increased infection rate in ticks is caused by: -An aggregation of larvae timed with maximum activity of infected nymph (and infected competent hosts) in July caused by the sudden increase in blood supply 1.5 - 2 months earlier. This will lead to increased rate of infected nymphs the following year because an increased part of larvae will quest in July-August. -The change in tick/host rate contributes to an increased infection rate by increasing the rate of infected competent hosts.

Isolation and partial characterisation of a species-specific 30 kDa-antigen from the third-stage larvae of *Oesophagostomum dentatum*

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Oesophagostomum dentatum, the nodular worm of pigs, is considered to be one of the most thoroughly adapted gastro-intestinal parasites. It causes lasting patent infections with only rarely occurring clinical signs of disease. Although antibodies against the parasite are produced, there is no significant protection. Former investigations into the immune response to this parasite have shown considerable differences between the antigenic profiles of different life cycle stages.

The aim of our study was to compare antigenic profiles of different *Oesophagostomum* stages and species in order to find specific antigens.

For this purpose we compared homogenates of free-living and parasitic stages of this parasite in Western blots and found the infectious third-stage larvae (L3) to be the ones that gave the strongest signals. A protein of about 30 kDa only appeared in the profiles of the free-living larvae, and most strongly in the one from the L3. Incubation with homologous and heterologous sera from monospecifically infected pigs revealed species-specificity of the protein in question. When *Oesophagostomum quadrispinulatum* blots were investigated the same way, they revealed a species-specific protein of similar size.

The *O. dentatum*-derived protein was partially purified using ion exchange chromatography and used in Western blots with various sera from experimentally *Oesophagostomum*-infected and helminth-naïve pigs, and the results showed the usefulness of this antigen as an indicator for serotesting of pigs.

Enniatins - a novel class of anthelmintics

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The 24-ring membered cyclooctadepsipeptide PF 1022A was recently isolated from the fungus imperfectus *Mycelia sterilia* and structurally characterized by Sasaki et al. (1992). Starting from this potent anthelmintic drug we have studied 18-ring membered cyclohexadepsipeptides such as naturally occurring enniatins like enniatin A, A₁, B, B₁ and beauvericin.

Comparison of these enniatins revealed that enniatin A shows an anthelmintic activity against *Nippostrongylus brasiliensis*, *Trichinella spiralis* and *Heterakis spumosa* at a concentration of 5 µg/ml in-vitro.

On the other hand beauvericin has only weak anthelmintic activity against nematodes in-vivo, respectively.

The structure of enniatins was stepwise modified using total synthesis. In our investigation it was found that especially (R)-lactic acid containing enniatins, e. g. JES 1798, have an anthelmintic efficacy against nematodes in livestock animals (sheep) in-vivo. The conformation analysis of 35 enniatin main conformers via 2D NMR techniques (H,H-COSY, NOESY, HMQC, HMBC) as well as molecular modelling studies clearly indicate a correlation between their conformation and nematocidal activity.

The radiolabeled enniatin [N-methyl-³H] JES 1798 showed an efficient and specific binding to a membrane fraction from the pig intestinal nematode *Ascaris suum*. Displacement by unlabeled JES 1798 was half-maximal at about 0.72 ± 0.06 mM. Different known enniatins also competed for the [N-methyl-³H] JES 1798 - binding in *A. suum* membrane preparations.

Alternatively, the preparation of (R)-lactic acid containing enniatins is possible by enzymatic biosynthesis.

These results indicate that 18-ring membered cyclohexadepsipeptides like (R)-lactic acid containing enniatins represent a novel class of anthelmintically active compounds.

Schistosoma japonicum and *Trichuris suis* infections in pigs fed diets with high or low protein

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The aim of the study was to measure the impact of *Schistosoma japonicum* and *Trichuris suis* infections in young growing pigs fed low- or high-protein diets. Thirty-two pigs, 6-10 weeks old, were randomly allocated to two groups receiving either a high- or a low-protein diet. After 11 weeks half of the pigs from each group were infected with 1,500 *S. japonicum* cercariae and 4,000 *T. suis* eggs. The weight of the pigs was measured throughout the study and blood and faecal samples were collected fortnightly from the time of infection. At the time of infection the low-protein pigs had significantly lower mean body weights, haemoglobin and albumin levels compared with the high-protein pigs and this pattern continued throughout the study. The serum albumin concentration was further significantly reduced in the infected low-protein pigs compared to the non-infected low-protein pigs. Significantly more *S. japonicum* worms as well as faecal and tissue eggs were found in the low-protein pigs compared with the high-protein pigs. No differences between the two diet groups were observed in *T. suis* establishment rates or faecal egg excretion. We conclude that this low-protein diet increased the establishment rates of *S. japonicum* and favoured larger deposits of *S. japonicum* eggs in the liver and faecal egg excretion, reduced weight gains and caused anaemia and hypoalbuminaemia in young growing pigs as compared with a high-protein diet.

Pentastomid infections in Nile crocodiles (*Crocodylus niloticus*) and cichlid fishes in the Kruger National Park

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Five pentastome genera belonging to the family Sebekidae and Subtriquetridae are known to infect the respiratory tract of crocodilians. Two Nile crocodiles were obtained from the Kruger National Park (KNP), a healthy specimen from the Phabeni Dam and another, in a severely debilitated condition, from the Shimuwini Dam. Both were males over 3m long and both harboured the three pentastome genera *Sebekia*, *Alofia* and *Leiperia* (Family Sebekidae). *Sebekia* and *Alofia* were recovered from the bronchioles, while female *Leiperia* occurred in the trachea and bronchi. Adult *Subtriquetra* (Family, Subtriquetridae) were not present in the nasopharynx of either crocodile.

Red-breasted bream, *Tilapia rendalli* (n=185) and Mozambique bream, *Oreochromis mossambicus* (n=119) were caught with baited handlines in the Phabeni Dam. The prevalence of infective pentastome larvae was 40.5% in *T. rendalli* and 9.2% in *O. mossambicus*. Sebekiids and subtriquetrids were collected from both fish species: a single encapsulated nymph of *L. cincinnalis* was taken from the mesentery, while *Sebekia wedli* were either encapsulated or free-living in the swim-bladder. The subtriquetrid larvae moved about freely in the swim bladder. *Sebekia wedli* was present in 2.5% of *O. mossambicus* while 7.5% were parasitised by *Subtriquetra*. This ratio was inverse in *T. rendalli* (40.5% and 2.2%, respectively). Of the infected *T. rendalli*, 89% harboured 1 or 2 sebekid larvae while a single fish harboured 8 larvae. The fishes infected with *Subtriquetra* contained 1 larva each.

Three young female crocodiles were infected with infective larvae obtained from the fishes collected in the KNP. After 226 days the crocodiles were sacrificed and adult *Sebekia wedli* found in one. One female was placed in phosphate buffered saline for one hour and produced 3 400 eggs. The eggs, 70% of which contained fully developed primary larvae, were concentrated to a final concentration of 68 eggs/ml water and offered to 8 guppies (*Poecilia reticulata*). Within 31 days two infective stages, both confirmed as being *Sebekia wedli*, had developed in one of the guppies, thus closing the life cycle of this pentastome species.

Ticks and tick borne diseases of central Tanzania

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Tick and blood surveys were carried out in the Lower Kihansi area in Iringa/Morogoro Regions of Central Tanzania, where a hydroelectric power plant is being established. The objective was to identify the tick species present in the area and the tick borne pathogens transmitted to man and animals. This was in response to complaints from non-indigenous people working in the area who got bitten by ticks and suffered serious reactions. The tick species identified from the area were *Amblyomma variegatum*, *Rhipicephalus appendiculatus*, *R. evertsi*, *R. kochi*, *R. pravus*, *R. sanguineus* and *Haemaphysalis leachii*. Efforts to isolate the pathogens from the ticks failed but serological tests by use of ELISA and IFAT techniques on sera collected from humans, baboons, gazelle, cattle, sheep, goats, pigs, chicken and rodents revealed the following pathogens to be circulating within the human and animal populations: protozoans: *Anaplasma*, *Trypanosoma* and *Cowdria*; rickettsia: *Rickettsia conorii*; viruses: Wesselsbron, Chikungunya, Sindbis and West Nile. This was a preliminary survey to provide baseline information. There is need of carrying out a more extensive survey in order to establish the extent of the problem, and also to associate the different pathogens to specific tick species so as to be able to design appropriate control measures.

Cystic echinococcosis in Morocco

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Cystic echinococcosis is a serious public health threat in the rural regions of Africa and the Middle East.

In Morocco, the annual incidence of surgical cases recorded from 1980 to 1992 ranged between 3.6 and 5.5. Individuals most affected were associated with agriculture and lived in rural areas. The prevalence of *Echinococcus granulosus* infection in dogs ranged between 23 and 58 %. Infection levels in intermediate hosts showed a range of 23-57 % in cattle, 1-26 % in sheep, 1-5 % in goats and 1-80 % in camels.

Factors responsible for the persistence of *E. granulosus* infection in dogs and high prevalence rates in man and domestic animals are a lack of general knowledge in the population at risk about the disease and its routes of transmission, large numbers of stray dogs and insufficient infrastructure and regulations in rural abattoirs.

The use of educational aids on schoolchildren in a highly endemic area of Morocco showed a significant increase in knowledge levels and indicated that specifically designed educational materials for all target audiences should be an essential component of any hydatidosis control program.

A compendium which compiles all data on the disease in Africa and in Middle Eastern countries, with special reference to Morocco, was produced by joint efforts of a group of scientists, and complimentary copies of the compendium will be available at the 1997 WAAVP meeting. The general hope is that control measures will be implemented in all countries where this disease is endemic.

Ticks associated with goats in the North-West Province of South Africa

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Tick (ixodid only) infestations of indigenous goats were monitored on a rural farm in Madikwe District of the North-west province of South Africa for a period of twelve months. Ticks were collected monthly from 24 study goats. The experimental goats were not dipped in acaricides throughout the study period. Different parts of the body namely, ears, hoofs, anus and others (head body, adder and tail tip) were examined. Samples from each part of the body were kept and preserved separately. Over six different species of ticks were identified and their populations fluctuated with the seasons with higher numbers in summer than in winter months. It was found that the ticks were very selective in choosing where to engorge. That is different species were found on different parts of the animal. The immature forms also preferred different parts from those selected by the adults. Serious alterations were caused on the parts of the body where heavy infestations of ticks occurred and led to some bleeding especially during the removal of ticks. It was observed that artificial engorgement sites were created where the experimental animals were tagged (on the ear lobes). The ticks in such areas were removed but were excluded in the sample.

Rhipicephalus e. evertsi and *Amblyomma hebraeum* were the dominant species. The larvae and nymphs of the former were almost exclusively found in the canals of the ears while the adults male and females engorged only round the anus in a 3:1 ratio. Both forms were completely absent from the hoofs and other parts of the body. *Amblyomma hebraeum* nymphs and larvae preferred the hoofs and sometimes the head and adder. Adult males and females also engorged round the anus. This selectivity in areas of engorgement in the different species makes identification, to a certain extent, predictable.

Although, it was not possible to quantitatively assess the actual physiological and other effects of the ticks on the animal health, the physical appearance and apparent loss of weight of the experimental goats compared to the regularly dipped one is an indication of the economic importance of ticks in rural areas where a farmer may not afford to dip his animals.

Please kindly note that due to technical problems this is not typed in a good scientific style. I am trying to 'catch the bus' despite all odds. Please let me know if I have a chance of sending one typed according to the prescribed rules next week when I return from the Eastern Cape.

Effect of storage on the pathogenicity and immunogenicity of *Eimeria tenella* oocysts

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The pathogenicity of chicken *Eimeria* spp. is said to be reduced due to storage in 2% potassium dichromate at refrigeration temperature, but the extent of this is not clearly understood. A single oocyst derived strain of *Eimeria tenella* isolated from Mymensingh, Bangladesh were studied to investigate the extent of reduction in pathogenicity due to storage, and its possible effect on the immunogenicity of the protozoa. Groups of twenty three -day- old chicks infected with different batches of stored and fresh oocysts (10^4 /bird) showed a different degree of pathogenicity with significantly different ($P < 0.05$ - $P < 0.01$) haematocrit values and weight gain. Oocysts were detected in the faeces of chicks on 6 day post infection (dpi) with fresh oocysts and 7 dpi with stored oocysts. The total number of oocysts produced from 6-12 dpi inclusive by the chicks infected with oocysts stored for different length of time were higher ($132 - 149 \times 10^6$) compared to the number (113×10^6) produced by the chicks infected with fresh oocysts. All the groups of chicks, except the group receiving oocysts stored for five months, immunised by two doses of 0.5×10^3 and 10^3 oocysts at 10 days interval developed a high degree of immunity both in terms of oocysts output and weight gain against a challenge infection with 10^4 oocysts 10 days after the second immunising dose. The results suggest that despite a degree of reduction in pathogenicity of *E. tenella* due to storage of oocysts in 2% potassium dichromate at refrigeration temperature, the immunogenicity is retained at least up to six months.

THE INFECTION BETWEEN TRYPAOSOME INFECTION AND PLANE OF NUTRITION

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The current study was conducted to evaluate erythropoietic responses in sheep infected with *Trypanosoma congolense* and given either a high (116g Digestible Crude Protein (DCP)) per day or a low (51.5g DCP/day protein diet). It was observed that dietary protein had no influence on the prepatent period and subsequent intensities of parasitaemia and also the rate of development and degree of anaemia that followed infection. However, it had an influence of rates of growth in that the infected and control animals on high protein diet grew at similar rates while infected animals on low protein diet experienced marked retardation of growth compared to their uninfected controls.

Ferrokinetic measurements indicated that infected animals had lower ^{59}Fe half-lives and estimated red cell lifespans but higher values for Plasma iron turnover rates (PIIR), ^{59}Fe utilisation and RBC ^{59}Fe incorporation rates than control animals. It was also observed that PIIR and ^{59}Fe incorporation rates were higher in the high protein infected group than in the low protein infected group although these differences were not significant. These observations indicate that the erythropoietic responses was enhanced in infected animals on high protein diet showed greater enhancement of erythropoietic activity than infected animals on low protein diet.

Anthelmintic resistance to gastrointestinal nematodes of sheep in Morogoro, Tanzania.

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A benzimidazole resistant strain of *Haemonchus contortus* in sheep in the Animal Science flock, Sokoine University of Agriculture (SUA), Morogoro, was suspected in 1986 and confirmed in 1990. Levamisole which had an efficacy of 100% at that time was recommended to replace benzimidazoles for deworming sheep.

After a period of about 10 years use of levamisole, this study was carried out to compare the efficacies of albendazole (vermectin) and levamisole (milvam) against gastrointestinal nematodes in sheep in the Animal Science sheep flock using Faecal Egg Count Reduction Test (FECRT). Three groups of Blackhead Persian sheep were randomly selected each comprising of 12 sheep. Group A served as untreated control, group B was treated with levamisole at 7.5 mg/ kg body weight and group C was treated with albendazole at a dose of 5 mg/ kg body weight.

Fourteen days post-treatment mean Faecal Egg Count Reduction of 95% with the 95% lower confidence limit of 85% were observed in group B treated with levamisole. In group C, treated with albendazole, a mean FECR of 91% and the 95% lower confidence limit of 69% was obtained.

These results indicate that gastrointestinal nematodes of sheep in the Animal Science flock at SUA are still resistant to benzimidazoles some ten years since it was withdrawn from use in the farm. Resistance to levamisole is suspected to have developed in gastrointestinal worms of sheep in this farm. The magnitude of anthelmintic resistance of gastrointestinal worms in sheep at SUA seems to be increasing with time.

COMPARATIVE STUDIES ON *SCHISTOSOMA SINENSIS* FROM NORTH THAILAND AND SICHUAN PROVINCE, CHINA

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In Southeast Asia, there are three species of human schistosomes: *S. japonicum*, *S. mekongi* and *S. malayensis* and five species of non-human schistosomes: *S. spindale*, *S. nasale*, *S. indicum*, *S. incognitum* and *S. sinensis*. The three human schistosomes are transmitted by the procercariae snails *Pomatopsidae*, while these species of non-human schistosomes except for *S. sinensis* are transmitted by the pulmonate snails *Planorbidae* or *Lymnaeidae*. In the case of *S. sinensis*, it is intriguing of all: the cercariae develop in *Tricula* which is placed in the family *Pomatopsidae*, the intermediate host of human schistosomes in Asia. Furthermore the egg possess a lateral spine much more resembles the eggs of *S. mansoni* than that of *S. japonicum*. As interesting possibility is that some character-states shared with both *S. japonicum* and *S. mansoni* are homologous, indicating that *S. sinensis* is derived from a common ancestor to both groups.

S. sinensis was first discovered in Mianzhu County, Sichuan Province, China on 1959. However the natural definitive host in this area has not yet been found. The parasite in China is little known and poorly understand. The presence of this parasite was also reported from North Thailand and the parasite have redescribed on the basis of adult worms and eggs from naturally infected final host (*Rattus rattus*) by Greer et. al (1989).

We carried out a survey on this parasite in North Thailand and Sichuan Province, China, and collected adult worms and eggs. Adult worms and eggs from both endemic areas were compared morphologically and are using as the materials for investigating in molecular phylogeny.



Development of a PCR-based assay for the identification of cyathostome species in horses.

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Cyathostomes are now recognised as the primary parasitic pathogen of the horse. Infection with these intestinal nematodes results in weight loss, colic and, in some cases, acute diarrhoea that can be fatal. Despite the widespread use of modern anthelmintics, disease due to cyathostomes is still common. There are several reasons for this: (i) cyathostomes can acquire resistance to benzimidazole drugs, (ii) when in arrested development in the intestinal mucosa, cyathostomes are highly refractory to most anthelmintic drugs and (iii) horses do not develop a strong acquired immunity to these parasites. Cyathostomes exist as a large subfamily consisting of over 40 different species which can only be identified by morphological examination of adult stages in the intestine. To enable diagnosis and identification of cyathostome species from egg and larval stages in faecal and pasture samples, a PCR-based assay of common species is being developed. Using DNA extracted from morphologically identified species, the ribosomal DNA (rDNA) intergenic spacer (IGS) region from cyathostomes has been amplified using primers designed from published sequences of the adjacent 26S and 18S rDNA genes of other organisms. Amplified fragments (~1.4kb) have been cloned and sequenced in order to identify regions of inter-specific divergence that may be effective in species differentiation.

The helminth parasites of African wild carnivores and their importance

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Although African wild carnivores have their own characteristic helminth parasites, they also share some of the parasites of domestic animals and some have zoonotic importance. Some of the parasites are acquired from devouring prey animals where the infective larval stages occur. Care should, however, be taken when identifying parasites through finding eggs in faecal samples because some of these eggs may be those of species specific to prey animals, such as eggs of schistosomes or amphistomes. The helminthic fauna is dominated by nematodes and, to a lesser extent, by cestodes. Digeneans are few and rarely encountered. Species of *Toxocara*, *Toxascaris*, *Ancylostoma*, *Necator*, *Uncinaria* and others have direct life cycles. Species of *Physaloptera*, *Gnathostoma*, *Cyathospirura*, *Rictularia* and others use insects or other hosts as intermediate hosts. Species of *Trichinella*, *Trichuris*, *Capillaria* and others also occur. The cestode fauna is dominated by the family Taeniidae particularly species of *Taenia* and *Echinococcus*. Adult species of *Taenia* have a fairly high degree of host specificity but their larval infective stages exhibit loose host specificity.

Several species have veterinary and public health importance. Species of *Ancylostoma*, *Necator*, *Trichinella*, *Taenia*, *Echinococcus* and others infect man and other animals either as larval forms or adults, and have great economic importance.

The swine gastrointestinal antibody response to whipworm infection

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The swine whipworm, *Trichuris suis*, is a parasite present throughout the United States and is of growing concern to the swine industry worldwide because it is very pathogenic to growing pigs. The economic threat posed by *T. suis* and other intestinal parasite infections has created a strong interest in the development of parasite vaccines for the swine industry. Use of a vaccine either alone or with anthelmintics should reduce the economic losses. However, before effective parasite vaccines can be created, the dynamics of the swine gastrointestinal immune response to parasite antigens must be understood. In this study, an enzyme-linked immunospot (ELISPOT) assay was developed to measure total and antigen-specific (AS) IgG and IgA antibody secreting cells (ASC) from gut-associated lymphoid tissues (GALT) [mesenteric lymph node explants from jejunal region of small intestine (SI-MLN) and cecum in large intestine (C-MLN); colonic lymph nodes (Co-LN); and jejunal and ileocecal Peyer's patches (JJ-PP and IC-PP, respectively)] and lamina propria from the proximal colon removed from *T. suis* infected pigs. The local antibody responses were compared to peripheral antibody responses found in the spleen and submandibular lymph nodes. Serum IgG levels to *T. suis* were measured by ELISA. The total IgG and IgA ASC frequencies for the spleen and the SI-MLN were relatively constant, whereas, the frequencies for the lymphoid tissues closest to the site of the infection, C-MLN, Co-LN, and IC-PP, peaked dramatically during the course of the infection. AS-IgG and AS-IgA ASC frequencies were higher in tissues closest to parasite infection (C-MLN, Co-LN, IC-PP and proximal colonic lamina propria). There were no AS-ASC found in the submandibular lymph nodes. AS- IgG ASC predominated in C-MLN and Co-LN, whereas, AS- IgA ASC were higher in IC-PP and lamina propria. The kinetics of serum IgG levels were similar to antigen-specific ELISPOT results. The ELISPOT assay provided valuable information on the localization and compartmentalization of the swine gastrointestinal immune response to *T. suis* which resides in the cecum and proximal colon. In the future, this may be used in monitoring gastrointestinal immune parameters of pigs inoculated with a *T. suis* vaccine.

Polymorphism of buccal capsule of *Cylicostephanus calicatus* (Looss, 1900) Cram, 1924 (Nematoda, Strongylidae)

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During the study of Cyathostominae having got after dissection of horse in Thoms Institute of vaccine and serum (Siberia) two forms of *C. calicatus* were discovered. They were differed from after the buccal capsule sizes and marked as *forma minor* (width of buccal capsule 24-30 µ, length - 36-42 µ) and *forma major* (width of buccal capsule 30-35 µ, length - 43-51 µ). Measurements and statistic analysis of 40 males and 37 females of both forms were made. After other morphometrical characters those forms are the same. In *Cylicostephanus goldi* also the presence of two forms differs from the dimensions of buccal capsule were noted.

Till now intraspecific diversity of Cyathostominae of horses is studied not enough. Species *Cylicostoma barbatum* Smit and Notosoediro, 1923 and *Cylicostoma ornatum* Kotlan, 1919 were described earlier. These species differed from *C. calicatus* and *C. goldi* after the size of buccal capsule and were considered as synonyms later. We intended there were morphological forms of *C. calicatus* and *C. goldi* described above.



ABSTRACT

Bovine tropical theileriosis due to *Theileria annulata* is widespread in the Sudan and the economic losses are heavy particularly among exotic and cross bred cattle. It constitutes a major obstacle to the development of milk and meat industry.

Calf mortality has been estimated at 30-40% and adult mortality about 20-30% in cross bred animals. Antibody surveys showed a prevalence rate of 46% in indigenous cattle and 84% in crossbred animals. The economic effects vary with the standard of hygiene, the nutrition status and the degree of tick challenge.

Hyalomma anatolicum anatolicum is the major vector of the disease in central Sudan. The disease vector in other parts of the country, outside the distribution of *H. a. anatolicum*, are *H. rufipes*, *H. impeltatum* and *H. dromedarii* and *H. truncatum* in the South and South Central areas.

Control of ticks and tick borne diseases using insecticides and chemotherapy are expensive. Immunization against the disease using the attenuated cell culture vaccine is recommended.

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The strigeoid metacercaria, *Diplostomulum xenopi* is commonly found in the pericardial cavity of the African clawed toad, *Xenopus laevis*. The objective of this investigation was to find and study all life stages of this parasite in the Free State, South Africa.

Cercariae, metacercariae and adults were studied using both light and scanning electron microscopy.

Metacercariae obtained from the pericardial cavity of *X. laevis* were fed to a laboratory-reared darter, *Anhinga melanogaster*. Eggs appeared in the faeces four weeks later and were collected and incubated at 28 °C. The miracidia hatched 14 to 19 days later and were used to infect the freshwater snail, *Bulinus tropicus*. Two generations of sporocysts developed, with the daughter sporocysts producing fork-tailed cercariae. The host was dissected and the adult worms were obtained from the duodenum.

After the morphological description of the adult and metacercaria, this parasite was taxonomically removed from the genus *Diplostomum* and re-allocated to the genus *Tylodelphys* in which the metacercarial stage was first described.

In the life cycle of *Tylodelphys xenopi*, *Bulinus tropicus* was thus found to serve as natural and experimental first intermediate host in the Free State. *Xenopus laevis laevis* was found to serve as natural and experimental second intermediate host and *A. melanogaster* as experimental final host.

Although the metacercarial stage was described in 1937, all attempts to find the final host failed. This might be due to possible host specificity of this species.

Efficacy of fenbendazole at an elevated dosage against cyathostome larvae within the mucosa

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A controlled trial was conducted to test the efficacy of fenbendazole at 10 mg/kg/day for five days against naturally acquired cyathostome infections. Twenty young ponies known to be infected were allocated into 2 groups of 10 each. One group received drug treatment and the other served as an untreated control. 3 of 10 ponies in the treated group and 4 of 10 in the control group were from a band shown by FECRT to be susceptible to FBZ at the normally recommended dosage (5 mg/kg). The remaining ponies were from a band shown to be resistant to FBZ. Using parasite recoveries determined by mucosal digestion and geometric means of total worm burdens, FBZ was shown to be highly efficacious against both hypobiotic L3 (90.5%) and developing L3 and L4 (95.9%). Efficacy against developing L3 and L4 was less if data collected using the transmural illumination method was considered (78.0%). This treatment protocol was equally effective against FBZ resistant and nonresistant cyathostome populations.

Experimental reevaluation of methods for the enumeration of mucosal cyathostome larvae

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The accurate enumeration of cyathostome larvae within the mucosae as it relates to the total worm burden of the horse is important for many types of studies. Variations of two methods or both have been used. Digestion methods are necessary to count the hypobiotic L3 stage (EL3). Transmural illumination (TMI) and/or DIG methods have been used to count the larger developing L3 (DL3) and early L4 stages. Experiments were conducted to directly compare these approaches. No differences were found in the numbers of DL3 or L4 counted from heavily infected nonanthelmintic treated ponies using either method. Numbers were lower following DIG than TMI of tissues from ponies treated with an effective anthelmintic. In cases of low level infections TMI is more sensitive. Freezing of tissues did not effect the counts of EL3 by DIG or DL3/L4 by TMI but the numbers of DL3/L4 were lower and more difficult to visualize following DIG. Fixation of DIG with 5% or 10% formalin, or glycerin ETOH did not effect counts. Clearly, both methods must continue to be used under some circumstances but time can be saved at necropsy by either freezing tissues or storing fixed DIG material for counting at a later date.



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The effects of a urea-molasses block supplement (UMB) on nematode infected Merino sheep offered a low quality roughage diet were investigated. Seventy-two 4-5 month-old Merino wethers were divided into 3 groups and fed either *ad libitum* oaten chaff and minerals (NU), pair fed to NU and offered UMB (UP) or fed *ad libitum* chaff and offered UMB (UA). Within each group 2 subgroups were infected *per os* with 200 *Haemonchus contortus* and 1000 *Trichostrongylus colubriformis* thrice weekly (INF) or remained uninfected (CON).

The UA sheep consumed more feed, gained more weight and grew more wool than the NU and UP sheep. In all dietary groups parasitism decreased feed intake, liveweight gain and wool production. Over time faecal egg counts (FEC) increased to high levels in all INF groups with NU > UA > UP. No difference was observed between the INF groups in total worm counts at slaughter. Protein and albumin concentration and PCV decreased over time in INF sheep with the greatest decline in NU, the least in UP while the UA sheep were intermediate. Infection decreased digestibility and H₂O and N intakes and retention while UMB increased H₂O and N intakes and urinary purine excretion.

Significant productivity benefits can be derived from supplementation of infected sheep with UMB but only if there is access to sufficient feed to fulfil additional requirements for dry matter intake resulting from increased ruminal efficiency. UMB without extra chaff reduced pathological effects of infection and reduced FEC but did not increase weight gain or wool production.

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The Danish Centre for Experimental Parasitology is involved in collaborative research projects on helminthosis in East and Southern Africa and in Nicaragua. As the applied aspects of the research have high priority, we have found it important to perform a large part of these studies on-farm. This is a strategy that has the clear advantage that the parasite problems are studied in their true environment. However there are also a number of challenges:

- The farm sizes are often small especially in integrated crop-livestock systems. It is therefore problematic to get statistically sufficient numbers of animals of a given age group for experiments. It is therefore necessary to include animals from several farms in one study.

- The farmers are often grazing their animals on communal grazing areas with several species and age groups grazing together. It is therefore difficult to implement or even to test strategic control practices.

- Control groups in the traditional sense are difficult to introduce in the traditional systems. To evaluate the impact of parasitism we are now working with a set-up where we keep a limited proportion of the animals (e.g. 25 %) practically worm-free through frequent or continuous. The difference between weight gain of these animals and the parasitized animals are used to estimate the impact of helminthosis.

For small-holders the most practical control methods seem to be tactical treatments of the most susceptible age groups combined with feed supplements to these groups. On-going projects are studying the epidemiology in different age groups, the impact of helminths and the effect of supplementary feeding.

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The Rock Elephant Shrew (*Elephantulus myurus*) is known as an important host for the immature stages of several tick species of veterinary importance. Notably amongst these is *Ixodes rubicundus*, the Karoo Paralysis tick. During investigation of the diversity and abundance of immature ticks on Rock Elephant Shrews many larvae and nymphs of *R. nutalli* were recovered from captured hosts. Adults of *R. nutalli* most commonly parasitize hedgehogs and porcupines but have been recorded from various other, mainly carnivore, hosts. Engorged nymphs, recovered from the captured hosts, were allowed to moult into adults which were fed on hedgehogs, *Atelix frontalis*. Females engorged over a period of 22 days and started laying eggs within 2 to 4 days. Egg laying reached a peak between 5 and 17 days and up to 996 eggs were laid per day. Eggs hatched after 58 days. Larvae and nymphs fed on *E. myurus* kept under controlled conditions and engorged in 4 to 7 and 4 to 15 days, respectively. The largest numbers of larvae (2 to 19 per host) and nymphs (4 to 8 per host) were recovered from captured *E. myurus* during August and September, with a distinct decline during the summer months. The results of this study expand our knowledge on the role of *E. myurus* as host to a wide repertoire of tick species.

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Ticks are very common in Martinique, more particularly *Boophilus microplus* (Canestrini, 1887). Feral dogs are very commonly found in pastures. To evaluate the exact role of these dogs in the dissemination of ticks on this island, a study was performed in 1995-1996. One hundred and one feral dogs were trapped and examined for ticks.

A total of 231 *Boophilus microplus* were collected on 12 feral dogs: 37 larvae, 152 nymphs, 17 males and 25 engorged females. Two dogs were highly infested: on one dog we collected 168 *B. microplus* (22 larvae, 119 nymphs, 10 males, 17 females), on another one: 44 *B. microplus* (11 larvae, 24 nymphs, 7 males, 2 females). Only 20 *B. microplus* were recovered from the others 10 trapped dogs.

Dog infestation by *Boophilus microplus* has never been described. This study allows us to assume that the dog is a physiologically suitable host (all the stages were found on them, including engorged females), but perhaps, it is not important in the maintenance of tick populations in a given geographical area. There is insufficient evidence to support arguments that a population of *B. microplus* can persist for years on dogs, because the development of the engorged females was not assessed.

Differences in growing rate and circulating IGF-I in ivermectin treated and nematode naturally infected dairy heifers from birth to puberty

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Growth and endocrine parameters were studied in dairy heifers from birth to puberty. Naturally infected and continuously ivermectin treated animals were compared. Twenty new born Holstein heifer calves were injected subcutaneously with Ivermectin (Ivomec, MSD AGVET, Buenos Aires Argentina, 0.2mg/kg BW) every 14 days until 150kg BW. At this time they received an intraruminal ivermectin bolus (Ivomec, 1.72g) which was renewed 120 days later. Twenty age matched calves received no treatment. Both groups were fed on a nematode infected pasture. Every 14 days animals were weighed and faecal and jugular blood samples were collected. Pelvic area was measured at 32 and 40 weeks of age and puberty attainment was evaluated by weekly progesterone samples. At 6 weeks of age ivermectin heifers were heavier than untreated ones (56.13 ± 1.21 vs. 52.08 ± 1.22 kg $p < 0.05$). This difference increased with age. Interestingly enough no nematode eggs were found in faecal samples until 12 weeks of age in both groups. After that age, *Cooperia* and *Ostertagia* eggs appeared only in the ivermectin treated group. Number of eggs increased until 20 weeks (873.8 ± 216.8 epg) and from then onwards decreased gradually. Serum IGF-I levels followed the typical developmental curve in both groups but ivermectin treated animals had significantly higher levels than untreated calves beginning at 16 weeks of age. The onset of puberty as shown by serum progesterone levels occurred earlier in treated heifers and pelvic area was higher at 40 weeks in this group (142 ± 3.97 vs. 125.11 ± 5.22 cm², $p < 0.02$). These results confirm the negative effects of parasitism on growth puberty onset and pelvic area. IGF-I is not probably involved in early differences observed in body weight gain but may affect pelvic development and puberty onset. Results also suggest that ivermectin may improve body weight gain in young dairy calves even before internal parasite eggs can be detected.

Nematophagous fungi as biological control agents against animal parasitic nematodes in integrated control strategies - An up-date

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In the past biological control (BC) of gastro-intestinal parasites in grazing animals has been considered as a marginal, 'exotic' research area, only interesting a limited number of scientists, but today BC is getting increasing attention around the world. In Central and South-America, where anthelmintic resistant nematode populations consist a major and increasing problem, several groups are engaged in project involving nematode-destroying fungi. Also, in Australia and New Zealand as well as in a number of countries in South-East Asia the potential of biological control of parasitic nematodes is being considered as a future adjunct to the present control strategies.

Within the past 5 years numerous trials have documented the potential of the so called nematode-trapping fungi, and in particular the species *Duddingtonia flagrans*, as BC agents against the free-living stages of parasitic nematodes in cattle, horses, sheep and pigs. Although only reports utilizing fungal spores as a feed additive or dosed via drinking water has been published, further work on development of feed-blocks and slow release devices should be strongly encouraged and supported. The fact that patents has been obtained on the BC principle plus at the same time improvements in yields and other developments of a fungal product progresses, the day when a commercial available product is ready appears to move a bit closer.

In the future the BC principle should be tested in different climatic zones under various management systems employed for the different species of grazing animals. Implementation of BC fungi in a number of selected management systems will be discussed. Also, the future needs for continuous comparative research on potentially new or improved BC candidates will be presented, discussing potential and possible limitations of various strategies.

Litomosoides spp (Nematoda:Filarioidea) parasitizing *Akodon cursor* (Winge, 1887) (Rodentia:Muridae) from Brazil.

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All animals, including man, may present multiple helminth infections. These have different destructive behaviors and interfere in the immune response of the host, making it difficult to diagnose the helminthic infections. Aiming to study experimental models for human filariasis, we trapped several wild rodents from the species *Akodon cursor* in the locality of Catimbau Grande, Rio Bonito, RJ, Brazil. They were naturally infected with various helminths, including filariids. Some of them presented, at the same time, two different species of filariids, occupying different habitats. The first species, that parasitized the thoracic cavity, was *Litomosoides silvaei* Padilha and Faria, 1977. The second was *Litomosoides chagasfilhoi*, that parasitized the abdominal cavity, a new species named in honor of Prof. Carlos Chagas Filho from the Universidade Federal do Rio de Janeiro, Brazil. Both were described according to investigations conducted by light and scanning electron microscopy. This was the first record of a rodent naturally parasitized by two species of filariids simultaneously.

Malignant theileriosis of sheep in the Sudan

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There are about 20 million sheep of different breeds and types in the Sudan. The majority are characterized by their big size, high growth rate and production of highly favourable mutton for local consumption and for export. Malignant theileriosis caused by *Theileria hirci* and transmitted by *Hyalomma anatolicum anatolicum* has emerged as an economically important tick-borne disease in northern, eastern and central Sudan. It causes severe outbreaks with high morbidity and high mortality rates. Epidemiological studies such as parasite transmission and isolation and breed susceptibility have been initiated.

The *Theileria* piroplasm parasitaemia in carrier sheep in the field was generally low (1/1000). The infection rate in field ticks with *Theileria* parasites was found to be low (1-2%). However, the feeding of *H. a. anatolicum* adult ticks collected as engorged nymphs from the field on susceptible sheep produced acute and fatal theileriosis infection. Schizont-infected lymphoblastoid cell lines were successfully established. Tissue culture schizont antigen and piroplasms antigen were prepared and used in indirect fluorescent antibody test. The parasite piroplasms stage in carrier animals can be confused with non-pathogenic species. Therefore, there is a need to develop a specific sero-diagnostic test. There is also a need to develop tissue culture vaccine and novel vaccines to immunize against *T. hirci*.



Monitoring of triclabendazole efficacy in naturally infected cattle using an antigen competition ELISA

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In Belgium, the liver fluke *Fasciola hepatica* mainly infects cattle. This pathology gives rise to anaemia, weight losses, low milk yields and poor beef production. Furthermore, liver are condemned following meat inspection.

Several chemicals are used to control this parasite with different success. Amongst all the products usually used, only triclabendazole is claimed to have 98-100 % efficacy on both immature and adult stages of *Fasciola hepatica* in small and large domestic ruminants.

The following study has been carried out in a massive infected herd as revealed by coprological examination of 55 dairy cows. This diagnostic was confirmed by both serological and antigen ELISA tests. Each animal was then treated with 1.2g/Kg of triclabendazole and regularly monitored by ELISA tests.

Results clearly show that the antigen competition test is convenient to follow more accurately the efficacy of flukicides than serological ELISA. This test is able to show a decrease of E/S antigens in the serum after one month while it takes more than three months to get the same trend with the antibody titres.

Nevertheless, some animals remain positive with this test. This positive response both for antigen and antibodies could be explained by the slow release of antigenic material from dead flukes encysted in liver tissue.

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Observations on fleece-rot and different types of blowfly strike in two districts in South Africa

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A flock of sheep comprising of 83 ewes and 100 lambs was monitored for a period of nine months for blowfly strike and incidence of fleece-rot in the Brandfort district (28°42'S ; 26°27'E), South Africa.

Only five sheep were struck by blowflies, while seven sheep showed signs of previous attack with loss of wool. Scouring was observed in 36 sheep, but no cases of fleece-rot were observed in the Brandfort district, but it was observed in the Verkeerdevelei district (28°50'S ; 26°47'E). Fleece-rot, a bacterial exudative dermatitis, develops as a result of prolonged wetting of the skin surface of the sheep and can result in body strike. Breech strike, due to urine or faecal stained wool in the crutch area, and poll strike, due to an abscess, was observed. Sheep showing break wool and loss of wool due to blowfly strike were also observed. An interesting case of blowfly strike due to a teary eye caused by the irritation of Annual Three-awn Grass (*Aristida* sp.) was also seen.

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The sheep blowfly problem in South Africa - an overview

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South Africa is amongst the largest wool producing countries in the world. The sheep blowfly, *Lucilia cuprina*, is one of the most important ectoparasites on sheep, due to great losses suffered by the wool and meat industries. An estimated R19.8 million was lost in South Africa during 1990.

During this study, South Africa was divided into three rainfall regions, viz 100-400mm, 400-700mm and 700-1300mm and questionnaires were distributed amongst sheep farmers. From questionnaires it was established that between 2% and 15% of wool sheep suffer blowfly strike annually, with less than 1% mortality. In all the rainfall regions blowfly strike peaks during the summer. Mainly ewes and lambs are effected by blowfly strike. On average R300 - R1000/year/farmer is spent on chemical control, but it could be as high as R5000/year. Due to urine wetting, the tail and hindquarters of sheep are mainly effected by blowfly strike in the low (100-400mm) and moderate (400-700mm) rainfall regions. In the high rainfall regions strike occurs on any part of the body due to a higher risk of fleece-rot. An average of 18% of the respondents indicated that they occasionally perform the Mules operation. However, it is generally not very popular in South Africa and is considered cruel.

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Chemical control of *Lucilia cuprina* under controlled conditions - the effectiveness of diflubenzuron and cyromazine

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Controlling methods of blowflies have changed significantly over the past years. As our knowledge of the ecology and biology of blowflies increased, recommendations for the control of sheep blowflies have changed. The use of insecticides is common amongst sheep farmers to prevent blowfly strike.

During this study, groups of treated and untreated sheep were exposed to *L. cuprina* in order to induce strike. Larval implantation was also used as a second method to test the effectiveness of the two insecticides. The treated sheep were plunge dipped in compounds with diflubenzuron (250g/litre) and cyromazine (500g/kilogram) as active ingredient. Experiments took place in a temperature controlled, fly proof sheep room. Under the conditions tested, it was evident that diflubenzuron and cyromazine will fully protect merino sheep against blowfly strike resulting from natural attacks for up to sixteen weeks. However, cyromazine gave better results during larval implantation from weeks 12 to 16 than during induced strike.

The acid-base and blood gas disturbances of severe South African canine babesiosis

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The acid-base and blood gas disturbances previously described in babesiosis include a metabolic lactacidosis and oxygen content deficit. Outcome has been demonstrated to be correlated to blood lactate levels. Arterial blood pH is not reflective of the severe base deficit frequently present. Treatment of the acidosis has traditionally been intravenous alkali (NaHCO_3) administration. Investigations have shown that acid-base and blood gas pathology can be reversed with whole blood transfusion. It has become evident that severe babesial disease is never associated with simple acid-base disturbances. In a detailed study of 15 cases the most consistent imbalances involved metabolic lactacidosis; hyperchloraemic acidosis and respiratory alkalosis. Similar changes have been reported in canine endotoxaemia, an experimental rat model of inflammation and in human sepsis. Just as human malaria has been likened to endotoxaemia, canine babesiosis has striking similarities. It is a clear example of the systemic inflammatory response syndrome which occasionally progresses to multiple organ dysfunction. Mortality is correlated with renal failure, lung failure and base deficit.

The oxygen dissociation curve is right shifted in canine babesiosis. This shifting is however compromised by the endogenous production of carbon monoxide, probably by haem-oxygenase salvaging of haemoglobin.

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DICTYOCAULOSIS IN DONKEYS IN KIAMBU DISTRICT OF KENYA

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The aim of this study was to identify the endo-parasites and the associated pathological lesions in naturally infected donkeys reared in Kiambu district of Kenya.

Two donkeys were slaughtered each in the month of April, June & August 1995 and a complete postmortem carried out.

In all the six donkeys, the lungworm *Dictyocaulus arnfieldi* was found in large numbers. Grossly, the lungs were pale, emphysematous and frothy. The larvae of the lungworms were found in the parenchyma whereas the adults were confined mostly in the air passages of the lungs. Histologically, there was exudation of leucocytes (Mostly eosinophils) and mucus into the air passages resulting in atelectasis. The unconsolidated areas of the lungs had emphysema.

Lungworm infection in donkeys has not been reported in Kenya and those reported elsewhere have not had severe pathological effects on the donkeys.

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Prevalence of common ruminant helminths observed in diagnostic faecal samples presented at veterinary investigation laboratories (VIL) Kabete, Kenya over a period of ten years (1984-1994)

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Helminth parasitism is a common occurrence in ruminants of all ages in the world, irrespective of the management practice. The infestation becomes important only if the productive performance is reduced and control should be aimed at minimising the levels of infestation so that production remains optimal.

Gastrointestinal tract helminths cause clinical and subclinical parasitic gastroenteritis in grazing ruminants and are still a major source of production loss in animal husbandry.

Parasitic gastroenteritis can also cause important production due to a lower growth performance, retardation of production activity, reduced milk production and the cost related to anthelmintic treatment.

The prevalence of common GIT helminths was assessed from faecal samples collected from large and small ruminant reported sick, and presented at the VIL Kabete, Kenya over a ten year period. Strongyle eggs were most prevalent followed by fascioliasis and tapeworm infestations.

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OCCURRENCE OF *IN UTERO* *BABESIA EQUI* CARRIER INFECTIONS IN THE OFFSPRING OF KNOWN CARRIER MARES

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Very little is known about how and why *Babesia equi* related abortions occur, or about the host-parasite interaction *in utero*. Classical theories suggest that once infected, the foetus is destined to be aborted. If one looks at other protozoan and rickettsial parasites it becomes obvious that this need not necessarily be the case. For example, bovines infected with *Anaplasma marginale* may give birth to clinically healthy calves which are parasite carriers. The trial reported on here aims to investigate whether a *B. equi* carrier state, similar to the *A. marginale* carrier state can be set up *in utero*, and to investigate whether the natural intake of colostrum has any effect on this infection. Six foetuses in different stages of gestation were mechanically aborted from known *B. equi* carrier mares. Polymerase chain reactions were carried out on DNA extracted from the foetal spleens. Amplification products were then blotted and probed with a radiolabelled *B. equi*-specific probe. A similar procedure was adopted with DNA extracted from blood collected from 7 newborn foals of less than 24 hours old. All foetal spleen and newborn foal DNA samples were positive for *B. equi*, indicating the presence of an *in utero* *B. equi* carrier infection. A *B. equi* carrier mare was placed in a tick-free isolation unit prior to parturition. Blood samples were collected from the foal prior to colostrum intake, and then daily for 21 days thereafter. The samples were analyzed for the presence of *B. equi* using the DNA probe technique. Parasite DNA was found to persist in the foal's blood for the entire 21 days of the trial.

Efficacy studies against fleas in pets with the new compound imidacloprid as compared to fipronil, diazinon and permethrin

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Objective: New effective, long lasting and easy to apply insecticides for control of fleas in pets are required in veterinary practice to overcome the increasing fleaproblems worldwide. Imidacloprid is a new active ingredient with high activity against fleas. The objective of this study was to compare the efficacy of the recommended dosage and application of three commercial products with the new compound imidacloprid against fleas in dogs and cats.

Methods: The study was conducted in accordance with the code of Clinical Trials on Veterinary Medical Products of the EU. Dogs (25 Beagle) and 20 cats were allocated to equal groups of five animals in which each animal was experimentally infested with 100 adult *Ctenocephalides felis* twice before and 6 times post treatment at weekly intervals. Cats in 3 groups were treated with imidacloprid, fipronil and diazinon, dogs with the same compounds and in addition a fourth group of dogs was treated with permethrin. One group of each animal species remained as untreated control. Fleas were counted at 24, 48 and 72 hours after reinfestation in dogs by using a comb. Eggs and adult fleas which were reared from these eggs were counted in cats.

Results: An immediate (24 hours) 100% killing effect was seen in all treated animals. In dogs 100 % efficacy was observed in all treatment groups until the evaluation after the third reinfestation. After the reinfestation 3 weeks post treatment (p.t.) only those dogs treated with imidacloprid and with diazinon were still free of fleas. On day 37 post treatment the reduction was 55% with permethrin, 83% with fipronil, 86% with diazinon and 99.8% with imidacloprid. In cats no flea eggs were produced 3 weeks p.t. with fipronil and imidacloprid, whilst 18 adult fleas emerged from pupae which were reared from eggs laid on day 17 after treatment with diazinon. The reduction of fleas reared from eggs laid on day 31 p.t. was still 79% with imidacloprid and 84 % with fipronil.

Conclusion: The trial results demonstrate the high efficacy of imidacloprid against fleas in dogs and cats when compared to other commercial products. The excellent knock down effect and the long lasting 100 % control of fleas together with the advantage of safety and simple application makes the product to a useful tool for flea control.

Coccidiosis in Springbok (*Antidorcas marsupialis*) in South Africa

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There is very little information about the effects of coccidiosis in wildlife. After several springbok died in a game farm, a carcass was brought to the Pathology Division at the Onderstepoort Veterinary Institute for post-mortem examination. Faeces were examined for the presence of coccidia oocysts and after 4 days of sporulation in 2 % potassium dichromate the oocysts were identified to belong to the genera *Eimeria*, but no literature reference was found to describe this parasite in the springbok. Lesions in the intestine were consistent with the pathogenesis of coccidia. Samples were collected from springbok in two different locations in South Africa and the morphology of this parasite is described. The use of different game species in the tourist industry in South Africa has increased the movement of animals to areas where they do not naturally occur. These stress factors increase the possibility of coccidia parasites being of considerable importance for this industry.

Tick infestation and serological evidence of simultaneous infection with *Babesia divergens* and *Borrelia burgdorferi* s.l. in cattle in Germany

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Objective: The tick species *Ixodes ricinus*, *Haemaphysalis punctata* and *Dermacentor marginatus* are the only 3 tick species known to infest cattle in Germany. Babesiosis in cattle is caused by *Babesia divergens* and by *B. major* which are transmitted by *I. ricinus* and *H. punctata* respectively. The occurrence of *Borrelia burgdorferi*, which is transmitted by *I. ricinus*, was questionable in cattle. However, since the vector for both pathogens is identical the occurrence of infection with both agents in the same animal could demonstrate the tick-borne genesis of *B. burgdorferi* s.l.-infection in cattle.

Methods: In 8 farms which were known for their enzootic infestation of cattle with *I. ricinus*, blood sera of 766 cattle were studied for infection with *B. divergens* and *B. burgdorferi*. The sera were tested by immunofluorescent antibody technique for *B.d.* and *B.b.* with antigens prepared from a German tick isolated *B.b.*-strain and a German isolate of *B.d.* in erythrocytes of *Meriones unguiculatus*. Cross reaction tests with 7 *Leptospira* spec. were included.

Results: We detected IFA titers against *B. burgdorferi* in 46.9% (359/766) of the cattle. While *Borrelia* infected cattle occurred in all farms the infection rates differed considerably from 4.4 % (2/45) to 90% (9/10). IFA titers against *B. divergens* were found in 1.3% (10/766) of the cattle sera. The infected animals were geographically restricted to 3 farms all of which had a known history of babesiosis. IFA titers against both pathogens were demonstrated in cattle from three farms. IFA titers against both pathogens in the same animal at the same time were seen only on one farm. On this farm, however, six of ten cows were simultaneously infected. All this animals were older than six years.

Conclusion: Cattle may become infected with *Babesia divergens* and *Borrelia burgdorferi* from bites of *I. ricinus*. In spite of the occurrence of the vector for both pathogens the infection rates with *Borrelia* spec. are much higher and the infection is geographically more widely distributed than the infection with *Babesia* spec. This may be explained by the occurrence of numerous vertebrates as reservoir hosts for *Borrelia* spec. compared to cattle as the only reservoir and host for *B. divergens*.

Strategic tick control on local zebu calves in Malawi Effects on Disease incidence in different ecological zones

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East Coast Fever one of the most important threats to cattle production in Malawi accounting for up 50% of calves deaths. Governmental tick control by compulsory weekly dipping failed to be effective due to poor management of dip tanks, shortage of acaricide and infrequent attendance to dipping.

A field trial was conducted in two different ecological zones, the Central plains region and the Lake shore area of northern Malawi. Tick control by „pour-on“ application of „Drastic Deadline“, (Flumethrine 1%) and by local application of „Tick grease“ (natural pyrethrines) was investigated. Effects on the tick burden, disease incidence and seroconversion to causal agents of Tick-borne diseases were studied. Newly born calves were monitored for one year. Seasonal changes in tick fauna and tick abundance were recorded. Blood slides and serum samples were analysed to confirm clinically apparent infection as well as inapparent infection mainly with *Theileria parva* and *T. mutans*. *Boophilus microplus*, *Amblyoma variegatum*, *Rhipicephalus appendiculatus* and *Rh. compositus* were found to be the most prevalent ticks. The occurrence of ECF in the Central plains region was highly seasonal with two peaks at the end of the dry season and in the mid-rainy season while there was evidence of endemic stability in the Lake shore area.

„Drastic Deadline“ proved to reduce tick burdens significantly for at least 2-3 weeks after treatment. Moreover ECF-incidence was reduced to approximately 50% by application in a 4-week-intervals (rainy season) and 8-week-intervals (dry season), respectively. Economic aspects and possible interference with endemic stability will be discussed.

Prophylactic *Psoroptes ovis* efficacy of Ivomec® SR Bolus in experimentally challenged Belgian White and Blue cattle

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Most outbreaks of psoroptic mange occur after winter housing. The IVOMEK (ivermectin) SR Bolus (ISRB) is designed to release ivermectin for 135 days.

Twelve Belgian Blue-White heifers were divided on body weight (185-328kg) into three equivalent groups. On Day 0, the start of the grazing season (June 26) all were treated with flumethrin pour-on (2 mg/kg). Group 1 heifers were controls; Group 2 and 3 were given an ISRB on Days 0 or 64 respectively. Animals remained on pasture until Day 153 (November 26) after which they were housed together for 13 days with two *Psoroptes*-infested seeders and then individually penned for 35 days (Day 202, January 13). Body weights were recorded on Days 0 and 167 (i.e. end of challenge) whereas mite counts and anti-*P. ovis* antibody titres were recorded monthly from turn-out to housing and weekly from Day 167 to Day 202. Mapping of lesions were performed on days 167 and 202.

The mean body weights were not statistically different between the different groups although the mean b.w. increase of Group 2 was higher. All skin samples were negative for *P. ovis* throughout the grazing season. After challenge, skin scrapings from Group 3 animals (Bolus on Day 64) remained negative whereas all Group 1 (controls) and 2 (ISRB administered 153 days earlier and challenged after ivermectin delivery has ceased) animals were positive for mite, with a good correlation with lesion scores and antibody titres.

The ISRB, administered later in the grazing season provided prophylactic efficacy against a heavy experimental *P. ovis* challenge at housing.

Efficacy of nitroscanate against cestodes and nematodes in cats, dogs and zoo animals.

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The purpose of this study was to investigate the efficacy and safety of the broad-spectrum anthelmintic nitroscanate in Lopatol 100^R and Lopatol 500^R. The 113 dogs and 48 cats and 134 zoo animals (family Felidae, Canidae, Ursidae and Hyaenidae) from Jihlava, Olomouc and Brno - zoological gardens in The Czech Republic. Animals of various breed, age, weight and sex with naturally acquired helminth infections (*Toxocara canis*, *Toxocara cati*, *Toxascaris leonina*, *Trichuris vulpis*, *Ancylostoma caninum*) and tapeworm (*Dipylidium caninum*, *Taenia spp.*) were treated with nitroscanate. Approximately 15 min. before treatment the animals were given 100 - 250 g of canned food. The single administration of nitroscanate in a dose 50 mg.kg⁻¹ was found to be a practicable and efficient method of anthelmintic therapy. The repeated application of 50 mg.kg⁻¹ in 24 h interval has increased the efficacy against *Trichuris vulpis* on more 92%. We determined the efficacy of nitroscanate to be 97,7 - 98,8% against *Toxocara canis*. The double application was more efficient 99,4% against *Ancylostoma caninum*, 99,2% against *Dipylidium caninum* and 100% against *Toxocara leonina*.

Acaricide resistance in ticks (Acari : Ixodidae) in Zambia

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Resistance to acaricides in *Rhipicephalus appendiculatus* (Neum.) *Boophilus decoloratus* (Koch) and *Amblyomma variegatum* (F) was determined in Central and Southern provinces of Zambia from 1990 to 1993 using the Food and Agriculture Organisation Acaricide Resistance Test Kit alongside locally prepared test papers. LC 50 (%) values for several acaricides used were determined for each species of ticks from hand drawn logarithm - probit graphs. Generally, resistance was developing in *R. appendiculatus* (traditional cattle sector) and *B. decoloratus* (commercial cattle sector dairy), with *A. variegatum* (traditional sector) being susceptible.

It was observed that engorged *R. appendiculatus* and *A. variegatum* ticks were easily obtained from traditional sector and were absent or in very low numbers as flat ticks on commercial sector beef cattle. Engorged *B. decoloratus* ticks from commercial sector were slightly larger and healthier than those from traditional sector cattle. The absence of engorged ticks and the difference in sizes of ticks in a specified farming system probably explains the roles played by the animal host and pasture management in limiting the survival of ticks.

Using either the FAO kit of locally prepared test papers one would get some pattern of data but probably with more details using locally prepared test papers which employ lower acaricide concentrations.

Ecological disturbance of endectocides on non-target fauna in pastures

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The endectocides (avermectins and milbemycins) comprise a group of highly efficacious, broad-spectrum anthelmintics, widely used throughout the world for the control of livestock parasites. Endectocides are excreted mainly in faeces, and to a large extent as unaltered drug. Because of this, these drugs have the capacity to exert adverse effects on insect populations associated with animal dung. Fears have been expressed that this reduction in fauna could have an adverse effect and potentially delay dung degradation, leading to fouling of pastures.

Three experiments with treated cattle, sheep and horses were designed to compare the effects of moxidectin and ivermectin on the fly *Neomysia cornicina* (Diptera) and the dung beetles *Aphodius haemorrhoidalis* and *A. constans* (Col. Aphodiidae). Larvae of Aphodiidae species and flies were bred in dung collected daily from day 0 to day D+38 post-treatment and the rate of emergence of adults was calculated.

In the cattle experiment, no significant differences were observed in the emergence of *A. haemorrhoidalis* fed with dung from controls or cattle treated with moxidectin (1% injectable solution, 1 ml per 50 kg b.w.) or ivermectin (0.2 mg per kg b.w.). For flies, the rate of emergence of *N. cornicina* with moxidectin was significantly lower than control until D+10 post-treatment, not different from control after D+16. The mortality was absolute (100%) until D+20 with ivermectin and the rate of emergence remained lower than control up to D+38. A series of dung pats were deposited in the field from day 0 to D+38 and recovered 2 months later. Compared with control, no differences occurred in the rates of dispersal whatever the treatment, even when endectocides markedly changed the fauna.

In the sheep experiment, a significant difference in *A. constans* rates emergence was observed between control and moxidectin for the first 2 days following treatment (0.1% oral drench at 0.2 mg moxidectin per kg b.w.). In the ivermectin group (Ivomec® oral drench at 0.2 mg ivermectin per kg d.w.), the differences with control were significant for 6 days post-treatment. Moxidectin was shown to be safe for *N. cornicina* development after 3 days post-treatment. In the ivermectin group, differences with control for *N. cornicina* were significant for 6 days, with null emergence for 5 days post-treatment.

In the horse experiment, a significant adverse effect was observed with *N. cornicina* for the first 5 days following treatment with moxidectin (2% equine paste at 0.4 mg moxidectin per kg b.w.), and for 21 days post-treatment with ivermectin (Eqvalen® equine gel at 0.2 mg ivermectin per kg b.w.).



Novel mRNA-direct cDNA cloning system for cloning eucaryotic cDNA: Rapid cloning of *T. colubriformis* cDNAs into vaccine vectors.

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Current methods for cloning eucaryotic genes, involve complex, labour-intensive procedures of constructing and screening cDNA libraries. Construction of cDNA libraries is expensive and subsequent to clone identification, involves multiple subcloning and sequencing steps before the insert can be suitably placed in the end-use vector e.g. for high-level expression of the target protein in vaccine delivery systems.

We have developed a novel technique which circumvents the need for cDNA libraries and downstream requirements of DNA sequencing and subcloning before the final end-use vector is obtained. In brief, the N-terminal amino-acid sequence of the target protein is determined and a designer-made, tailed-degenerate oligonucleotide is synthesised. It is used in a set of novel PCR reactions to amplify the complete cDNA encoding the target protein from the eucaryotic mRNA. The cDNA is then cloned into the final end-use vector in a precise manner. The precision cloning is made possible by the use of our designer tails for the PCR oligonucleotides. The cDNA is cloned in-frame into the final vector for the desired protein expression.

The procedure is simple to use, reproducible, cost-effective (approximately 5% of cost of cDNA library screening procedure) and reliable, eliminating the uncertainties associated with current procedures. The new process is the subject of a patent application.

We have isolated new extracellular/secretory antigens from nematode gastro-intestinal parasite of sheep, *Trichostrongylus colubriformis*.

Two of these antigens have been cloned into expression vectors demonstrating the success of our new cDNA cloning system.

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EPIDEMIOLOGY OF GASTROINTESTINAL HELMINTHS IN COMMUNAL CATTLE AND EFFECTS OF TREATMENT AND FEED SUPPLEMENTATION ON GROWING ANIMALS

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Two experiments are being conducted (a controlled study and a field study) to determine the influence of dietary protein supplementation and anthelmintic treatment in young growing cattle from the communal areas. For both studies, experimental animals with already established infections were randomly selected and divided into 4 groups: (1) treatment and supplementation (2) treatment only (3) supplementation only (4) control (no treatment and no supplementation). Panacur SR (slow release) boluses are being used in the treatment groups during the dry and wet seasons. Cotton moth and sunflower seed cake are given to the supplementation groups only during the dry season at calculated daily rations. The following parameters are being used to compare the performance among the 4 groups: egg counts, blood parameters (PCV, Hb, TP, Alb) and weight gain. Blood and faecal samples collected monthly for analysis and body weights recorded fortnightly. Treatment in both groups led to a significant decline in strongyle egg counts. Mean PCV, Hb, TP and Albumin were relatively higher in the treatment groups than in the non treated groups. Supplementing with cotton moth resulted in a higher mean weight gain in the treated groups than in the non treated groups. The supplementation results were however not conclusive, it has therefore been decided to repeat the experiment with a different supplement i.e. sunflower seed cake.

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Revalidation of *Physaloptera bispiculata* Vaz and Pereira, 1935 parasite of *Nectomys squamipes* Brants, 1827

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Species of the genus *Physaloptera* Rudolphi, 1819 (Nematoda : *Spiruroidea*) were recorded as parasites from the stomach of vertebrates, sometimes in the intestine (Ortlepp, 1937). *P. bispiculata* was described from *N. squamipes* (Rodentia : *Cricetidae*) from São Paulo, Brazil. It was also referred in three other rodent species (Morgan, 1941). Based in some similarities, Morgan (1943) proposed that *P. bispiculata* should fall as a synonym *P. getula*.

The present study was undertaken to clarify several aspects of morphology and taxonomy of the *Physalopteridae* parasite of *N. squamipes*. We have examined the type material, although fragmented, we recognised some of the features drawn by Vaz and Pereira (1935), as the spicules and the position of the papillae.

B. bispiculata collected during our experiments and examined optical and scanning electron microscopy was morphologically and morphometrically similar to the type series and belong to the same microscopy added several morphologic aspects of the anterior end, and detailed aspects of the aspects of the posterior end of the male and female adult worms. Comparative analyses of *P. getula* Scurat, 1917 *P. bispiculata* original descriptions, the considerations of Morgan (1943) and the present study, show that *Physaloptera bispiculata* Vaz and Pereira, 1935 is a valid species.

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Control of tsetse and animal trypanosomosis using a combination of tsetse trapping, pour-on and chemotherapy along the Uganda-Kenya border

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A joint tsetse and trypanosomosis control campaign has been going on along the Uganda-Kenya border since July 1991. A combination of tsetse trapping using insecticide impregnated pyramidal traps, pour-on and chemotherapy was used. Implementing departments in Uganda and Kenya harmonized tsetse and trypanosomosis control strategies used on either side of the border. Monitoring of the prevalence of trypanosomosis and tsetse population was carried out from January 1992 to September 1996. The area was divided into zones: A, B and C. In zone A, initially large scale application of Spoton was done then followed by trapping and selective chemotherapy. In zone B, small scale application of spoton was done followed by large scale block treatment of cattle and trapping. In zone C, trapping and initial block treatment of livestock was done. A total of 1220 head of cattle were screened every three months and tsetse apparent density monitored on a monthly basis. Between July 1991 and March 1994 the prevalence of trypanosomosis and tsetse apparent density in zone A reduced by 94 % (6.7 % → 0.4 %) and 99.5 % (0.55 → 0.003 F/T/D), respectively. Whereas, tsetse apparent density was maintained low between March 1994 and September 1996, the prevalence of trypanosomosis increased to 4.5 % and later reduced to 2.6 %. In zone B, between July 1991 and December 1995 the prevalence of trypanosomosis and tsetse apparent density reduced by 89 % (12.6 % → 1.3 %) and 99.3 % (0.44 → 0.003 F/T/D), respectively. However, between December 1995 and September 1996 the prevalence of trypanosomosis and tsetse apparent density increased to 10.6 % and 0.07 F/T/D, respectively. In zone C, from July 1991 to September 1993, the prevalence to trypanosomosis and tsetse apparent density both gradually reduced by 79 % (17 % → 3.6 %) and 95 % (0.65 → 0.03), respectively. From September 1993 to March 1995 both disease prevalence and tsetse apparent density were maintained low. Tsetse apparent density was maintained between 0.003 and 0.02 F/T/D upto September 1996 but the prevalence of trypanosomosis increased from 3.6 % to 18.6 % during the same period. The most prevalent trypanosome species during control in the different zones were: *T. vivax* in zone A, *T. vivax* and *T. congolense* in B and *T. vivax*, *T. congolense* and *T. brucei* in C. *Glossina fuscipes fuscipes* was the only tsetse species caught.



Prevalence of helminth parasites in free range chicken in Morogoro, Tanzania.

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A study was carried out to determine the prevalence of helminth infections in free range chicken in Morogoro, Tanzania. Matched samples of 100 growers and 100 adults were purchased from Changa village during the rainy season from February to May, 1996. At necropsy the trachea, gastro-intestinal tract and oviduct were examined for helminth infections.

The overall prevalence was observed to be 100%. A total of 27 species were isolated of which 19 were nematodes and 8 cestodes, no trematode was isolated. *Tetrameres fissipina* is reported for the first time. Each chicken was found to harbour more than two species. The growers had an average of 8.4 ± 2.1 with a range of (4-14) while adults had 7.3 ± 2.2 with a range of (3-12) helminth species. The prevalence of the following species was significantly higher in growers than in adults: *Ascaridia galli* (69% in growers, 29% in adults), *Tetrameres americana* (94%, 82%), *Trichostrongylus tenuis* (43%, 7%), *Syngamus trachea* (14%, 3%) and *Raillietina tetragona* (36%, 21%) respectively. While the prevalence of the following species was significantly higher in adults than in growers: *Allostapha suctorica* (19% in adults, 2% in growers), *Subulura strongylina* (8%, 1%) and *Capillaria annulata* (10%, 1%) respectively.

Student t-test about geometric means showed higher worm burdens in growers than in adults at different significant levels: *T. tenuis*, *S. trachea*, *Davainea proglottina*, *Capillaria contorta*, *Capillaria caudinflata* and *Heterakis isolonche* ($p < 0.0001$), *R. tetragona* ($p < 0.001$), *Choanotaenia infundibulum* ($p < 0.01$), *T. fissipina* and *A. galli* ($p < 0.05$). A positive correlations (Pearson) of log-transformed worm counts between species revealed that the following worm species had a tendency of occurring together: *Capillaria anatis* and *Capillaria obsignata*, *Capillaria bursata* and *C. obsignata*, *C. anatis* and *C. bursata*, *C. bursata* and *Amoebotaenia cuneata*, *T. americana* and *T. fissipina*, and *C. obsignata* and *A. cuneata*. This study showed high prevalence and high worm counts of some pathogenic helminth species in growers than in adult chicken. It is therefore, recommended that when designing a helminth control programme in free range chicken more emphasis should be paid to the growers.

Strategic use of moxidectin and closantel in combination with levamisole in the control of benzimidazole-resistant nematodes of sheep in Kenya.

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Moxidectin and closantel in combination with levamisole were compared in the strategic control of benzimidazole-resistant nematodes of sheep on a farm in Nyandarua District of Kenya. Thirty Corriedale female lambs aged between 6 and 8 months were assigned to three treatment groups of 10 lambs each. The three groups were setstocked on separate paddocks. Lambs in group 1 were dewormed with moxidectin at 0.2mg/kg and those in group 2 with closantel at 10mg/kg together with levamisole at 7.5mg/kg. These treatments were given strategically 3 weeks after the onset of both the short and long rains and at the end of the long rains season. The third group of lambs remained untreated (control group). Gastrointestinal nematode infections and pasture infectivity were well controlled in the strategic treatment groups. This resulted in higher weight gains, wool production and PCV compared with the untreated control lambs. These parameters were comparable between the lambs treated with moxidectin and those treated with closantel and levamisole. It was concluded that worm control strategies based on the epidemiology of the parasites and the sustained anthelmintic action of both moxidectin and closantel can provide effective control of gastrointestinal nematodes of sheep in the study area.

Pyruvate production method as an indicator of drug resistance of *Trypanosoma evansi* isolates.

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Drug resistance in camel trypanosomosis, which has been noted to be on the increase, needs to closely monitored. *In vitro* assay systems have provided an alternative to the conventional *in vivo* animal experimentation. However, the major disadvantage of this innovative technique is the reliance on culture adaptation of the trypanosomes. The aim of this study was to optimise the Pyruvate production method for use in the unadapted field isolates of *T. evansi*. The optimum conditions were found to be; field isolates from the infected camels passaged twice in mice, transfer of trypanosomes collected during the first phase parasitaemia to axenic culture media followed by an initial incubation period of two hours at 37°C and 5% CO₂. The harvested trypanosomes seeded at $1-3 \times 10^5$ trypanosomes/ml and incubated for a further 48 hours. The method was found to be sustainable for screening of resistance to the commonly used trypanocides (melarsomine, quinapyramine sulphate and suramin). It was reproducible, rapid, inexpensive and well suited for mass screening. This study recommends the use of the pyruvate production method in assessment of drug resistance in *T. evansi*.

Natural challenge with *Schistosoma bovis* and *Fasciola gigantica* in calves after treatment of the primary infections with praziquantel and triclabendazole.

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An experiment was carried out to determine the effect of challenge infections in calves treated with praziquantel and triclabendazole against primary *S. bovis* and *F. gigantica* natural infections in an area of high transmission intensity in Lulanzi village, Iringa district, Tanzania. Thirty five bull calves 6 to 9 months old, free from trematode infections were divided into five weight matched groups (A B C D E). Four groups (A B C E) were introduced to the farm in October, 1993. Monthly body weights, packed cell volume (PCV), haemoglobin (Hb), *F. gigantica* and *S. bovis* eggs per gram of faeces (EPGF) were recorded. Group D was kept free from infection. When all the calves, in groups A, B, C, & E, were positive for both parasites, calves in groups A&B were treated with both praziquantel (60 mg/kg bwt) and triclabendazole (12 mg/kg bwt). After treatment, group B (treated) and E (untreated) were removed from the transmission site and necropsied 6 weeks later. Group A (treated) and C (untreated) continued grazing and group D was introduced to check for post treatment transmission. Groups A, C and D were necropsied 11 months post-treatment. During postmortem examination, worm burden and tissue egg count (EPGT) were determined. Calves in group B had significantly ($P < 0.05$) reduced *S. bovis* faecal epgf (98.9%), *S. bovis* worm burden (95.3%), *F. gigantica* worm burden (100%) and *S. bovis* tissue egg count (small intestine=91-96%). No overt side effects were observed in all the treated calves. Calves in group A (treated) had significantly lower *S. bovis* EPGF than the untreated calves (group C) for most of the experimental period. However, at the end of the experiment both groups A and C had comparably low EPGF as opposed to the challenge control group D, suggesting that treatment did not affect the development of immunity. The untreated calves (group C) had significantly ($p < 0.05$) higher number of *F. gigantica* worms (mean=17) and *S. bovis* worms (mean=1332) than the treated calves (group A) which had mean worm counts of 6 and 504 respectively. There was no significant difference ($P > 0.05$) in the establishment of *S. bovis* worms between the treated group A and challenge control group D, suggesting that treatment did not prevent reinfection and establishment of the parasites. There was slightly high number of EPGT in the control than in the treated calves. After treatment and challenge, the untreated group C had lower body weight gains, PCV and Hb than the treated group A. It is concluded from the present study that treatment of early patent primary *F. gigantica* and *S. bovis* infections in cattle reduces morbidity as manifested by lowered worm burden, tissue and faecal egg counts. Apparently there were also long term positive effects on body weight gain and haematological parameters without interfering with development of immunity.



Individual vs pooled faecal worm egg counts (FEC)

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The aim of the investigation was to compare pooled and individual FECs for cutting costs and making the egg counts more practical for the practitioner. A modified McMaster method was used, at a sensitivity of 67 FEC, using sugar solution for flotation, and Visser Tube Filters to facilitate the counting.

INDIVIDUAL COUNTS: On 3 occasions over a period of 10d FECs were done on faeces from 110 sheep in 11 groups, heavily infected with *Haemonchus contortus*. After the first FEC 10 groups were dewormed with various anthelmintics, with reductions of 85-100%. The mean FEC of the 110 sheep was 14700 at the start.

POOLED COUNTS: At each occasion 1g of faeces from each of 5 randomly selected sheep per group was pooled for a "pooled-5" count, and so also from all 10 sheep ("pooled-10") in the group.

The mean difference (individual vs pooled FECs) was only 8% and 4% for the pooled-5 and -10 counts respectively, and the corresponding coefficients of variation were 405% and 685%. Respectively 44% and 56% of the pooled FECs were distributed above and below the means of the individual counts, with 50% of the pooled counts falling between +20% and -20% of the means of the individual counts, and 68% between +30% and -30%.

Considering the limitations of the FEC technique and the large variations in FEC invariably found in flocks naturally infected with *H. contortus*, we conclude that FECs of pooled samples are mostly similar in applicability to the means of 5 or 10 individual counts. On the other hand, individual FECs are probably better indicators of the level of husbandry and management on the farm, e.g. correct drenching of every sheep. Early drenching failure owing to resistance often presents as particularly large variations between individual sheep in a flock.

Coproantigen detection in dogs experimentally and naturally infected by *Echinococcus granulosus* using a monoclonal antibody-based enzyme-linked immunosorbent assay

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Parasitological diagnosis of *Echinococcus granulosus* in dogs is either non-specific, of relatively low sensitivity or hazardous. A sandwich ELISA for the detection of *E. granulosus* coproantigen in formaline treated fecal supernatants of dogs was developed. The assay used catching affinity-purified polyclonal antibodies obtained from rabbits hyperimmunized with excretory/secretory antigens produced by *in vitro* maintained prepatent adult *E. granulosus* and biotinylated monoclonal antibody Em9 produced against adult *E. multilocularis* somatic extract.

Thirteen helminth-free dogs were infected with different amounts of *E. granulosus* protoscoleces and the presence of coproantigens was monitored during the prepatent period until day 35 when they were necropsied. Fecal antigen levels started to rise above normal range between day 10 and 20 postinfection, and typically peaked at the end of the experiment. All the dogs, bearing from 3 to 67,700 worms showed ELISA positivity (100% sensitivity) at some time point of the prepatent period. One dog experimentally infected with *Taenia hydatigena* metacestode and harboring 3 worms, resulted positive only after the prepatent period at day 52. The test was applied to 101 stray dogs euthanased as part of the National Control Programme. The ELISA detected 4 of 4 dogs natural infected by *E. granulosus*.

Extended genetic resistance to strongylid species in a polish long wool sheep.

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An experiment was conducted from 1991 to 1994 in a sheep breeding unit from south Poland to estimate the genetic parameters of resistance to gastrointestinal nematodes and to define the specificity of resistance. The predominant species were *Teladorsagia circumcincta* and *Haemonchus contortus*. A total of 32 sires were evaluated, 14 per year, and the faecal egg counts (FEC) measured two times during the 4 months of grazing lambs and 323 ewes were sampled a year or more. Infection levels were high the two first years and low the two last ones. By using an animal model, the heritability estimates of FECs increased from the beginning to the end of the grazing, reaching values of about 0.30.

At the end of the grazing season in 1993 and 1994, 12 lambs issued from 2 resistant rams and 12 from 2 susceptible ones (plus 12 from 2 partly resistant rams in 1994) were necropsied. Lambs issued from susceptible rams had 1.5 to 2 times more worms than those issued from resistant ones, those from partly resistant rams being intermediate. Each lamb was infected by 2 to 9 species, but the proportions of the different species were similar between the 3 groups of lambs; the non specific mechanisms of resistance are genetically determined.

These results confirmed the feasibility of sheep breeding by adding an index on genetic resistance to nematode parasites in a long wool breed type and in an environment *T. circumcincta* and *H. contortus* were the dominant species.

Satellite Surveillance in veterinary parasitology

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An array of multispectral sensors on earth observing satellites provide new ways to map the earth and characterize the environment from space. Commercially available satellite image data can now be used with geographic information systems (GIS) software, digital map archives and geographic positioning systems (GPS) to provide, at the personal computer level, a powerful new tool for control of animal and human disease. Applications to date include malaria, *Schistosoma*, *Fasciola*, *Onchocerca*, *Dracunculus*, *Wuchereria*, African trypanosomiasis, *Leishmania*, Rift Valley fever, tick borne disease, foot and mouth disease and geohelminths. Many of these focus on mapping the distribution and abundance of disease agents and their vectors by matching the life cycle requirements to key environmental determinants. Others aim at simply using an easily updated dynamic records system on disease in which central office and field personnel interact with identical map databases. Within a GIS, satellite image analysis products can be used individually, as a time series, or as composites as a current map backdrop for point, line or polygon (area) data on the disease and other data such as soils, climate and thematic government maps and statistics. GPS allows placement of disease records to within 40-50m of occurrence. Complex Excel, Dbase or SQL databases can be linked to features on the map as GIS 'attribute data'. Taken together, these new technologies offer a better way to use maps in control programs. Satellite image availability, hardware-software requirements, GIS/GPS methods, digital map archives and ways to link GIS disease control models running on personal computers with larger central resource sites will be illustrated using examples from the literature and recent work on snail borne disease.

Assessing risk of *Fasciola* and *Schistosoma* : Geographic models, climate and coproantigen diagnosis

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Geographic information systems (GIS) and sensor data from earth orbiting satellites provide new ways to map environmental factors that influence the distribution and abundance of disease. Concepts developed for earlier climate forecasts and soil-hydrology GIS models for *F. hepatica* in cattle in Louisiana were used to construct environmental risk assessment models for schistosomiasis in Egypt and fasciolosis in East Africa. Diurnal temperature difference (dT) and vegetation indices (NDVI) were calculated using the AVHRR sensor from NOAA environmental satellites and used to describe thermal-hydrology domains in the Nile delta that correlate with prevalence of *S. mansoni*. A *Fasciola* forecast system based on daily climate data, water budget and the growing degree day concept was adapted for broader application by use of monthly data. FAO monthly climate data, a 10-year NDVI time series and crop production system zones databases for East Africa were used, in a GIS, to describe the overlapping distribution and abundance of *F. gigantica* and *F. hepatica* in highland-lowland regions. Stable 26kD coproantigens of *F. hepatica* found in western blots of fecal supernates were also detectable by antigen-capture ELISA. In cattle with known fluke burdens, ELISA was more accurate than fecal egg counts for detection of infections with >10 flukes.

Diminazene tolerant *Trypanosoma brucei rhodesiense* from Tororo, southeastern Uganda

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The study aimed at establishing whether *T. b. rhodesiense* parasites respond to veterinary drugs which would indicate whether the human infective parasites in the animal reservoir are effectively controlled by administration of veterinary drugs to animals. *Trypanosoma brucei* isolates from domestic animals were grouped with respect to the human serum resistance test. The humans serum resistant ones were denoted *T. b. rhodesiense* and the susceptible ones *T. b. brucei*. The former were detected in dogs, pigs and cattle which emphasised that animals harbour human infective trypanosomes and act as reservoirs in southeastern Uganda. After classification as *T. b. brucei* or *T. b. rhodesiense*, various stocks isolated from animals or humans were investigated for their susceptibility to the veterinary drugs diminazene aceturate or isometamidium chloride. The results indicated that no significant differences in drug sensitivity occurs between *T. b. rhodesiense* and *T. b. brucei*. It was also found that *T. b. rhodesiense* encountered in the animal reservoir responds to veterinary drugs. However, one *T. b. rhodesiense* stock isolated from a sleeping sickness patient showed a reduced susceptibility to diminazene aceturate and isometamidium chloride. Two hundred fifty times more diminazene or 78 times more isometamidium was required to eliminate that stock during 10 days of drug exposure in vitro, as compared to the most susceptible stock in each case. The in vitro drug responses of 4 sub-clones derived from this stock revealed that it was a collection of multi-drug-tolerant individuals. Treatment of mice earlier (72 hours) infected with the stock revealed that it was refractory to 3.5, 7.0 and 14.0mg/kg diminazene aceturate with 80 %, 60 % and 26 % of the mice respectively becoming patent. In contrast, treatment of mice infected with *T. b. brucei* which was shown to be drug sensitive in vitro practically effected permanent cure at all doses of both drugs. However, when cattle infected with this stock, or the sensitive stock or a mixture of the two were treated with the currently employed 7mg/kg dose in southeastern Uganda, the infection was cleared in all the animals. The implications of these findings to the control of trypanosomiasis are discussed.

Molecular characterisation of drench resistance in the gastrointestinal parasite *Ostertagia* in sheep

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Drench resistance in helminthic parasites is a major concern to the agricultural industry causing huge losses in stock production. A new technique called Representational Difference Analysis (RDA) is being evaluated as a means to locate and amplify possible genetic mechanisms that give rise to the trait of resistance in the sheep gastrointestinal parasite, *Ostertagia*. The system was firstly optimised in our laboratory on two isolates of *Giardia*, 12c14 and P1c10 which yielded a series of difference products. RDA was subsequently applied to two isolates of *Ostertagia*, one resistant to benzimidazole and levamisole, the other having had no exposure at all to either drug. Altogether, five difference products have been isolated and cloned into a pGEM vector for further analyses such as sequencing and southern blotting. It is hoped that this course of research will show that one or more of the difference products is associated with conferring resistance to the organism. If successful, the same course of research will be applied to other isolates of *Ostertagia* that have exhibited signs of resistance to macrocyclic lactone drugs, namely ivermectin and moxidectin. Resistance to these anthelmintics is emerging as a major threat as no new anthelmintics have yet been developed.

Prevalence and biodiversity of helminth parasites in donkeys in South Africa

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Approximately eleven million people live in the rural areas in South Africa and lack dependable and affordable transport. The use of donkeys for traction fulfil many daily needs for these communities e.g transport water and food and also agricultural practices. Little is known on the nematode parasites of South African domestic equids particularly donkeys. Donkeys are host to large numbers of helminths, many of which adversely affect their health and productivity. This study aims to inventory the biodiversity of worm species harboured by the donkey and illustrate their complexity. Total worm recoveries of the gastrointestinal tracts and organs of donkeys from the North West Province were studied, the helminths identified and their numbers estimated. Studies such as this are essential in the development of cost-effective and appropriate recommendations of control to reduce worm disease in working donkeys.



Sites of attachment and total counts of ixodid ticks (Acari: Ixodidae) on impala (*Aepyceros melampus*)

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During 1994 and 1995 45 impala ewes, *Aepyceros melampus* were examined at Letaba Ranch situated in the Northern Transvaal, South Africa. Tick counts were made from 15 animals during July, October and February. The objective was to determine whether total body tick counts can be estimated from counts done on specific, selected sampling sites on the skin. Ticks were collected, using a shaving technique from 12 sites on the body and compared to a control which was the total count as determined by two destructive sampling techniques. Six tick species were collected of which *Boophilus decoloratus* was the most abundant. Two mathematical models were tested. The first model was made up of tick counts from a single region on the animal's body, namely the pinna, the correlation between tick counts on the pinna and the total counts was highly significant. A second model based on tick counts from the head, the pinna, the front and hind foot was found to be less significant.

Non-specific reaction of equine leukocytes towards *Cyathostominae* infecting larvae (L3)

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Antibody dependent cell mediated cytotoxicity (ADCC) has been demonstrated against several parasites and is postulated as protection mechanism. In order to study protection phenomena against cyathostomes in horses, the interaction of equine leukocyte with L3 *Cyathostominae* larvae was studied in the presence and absence of serum. Larvae were obtained by coproculture. They included several cyathostome species and were uncontaminated with other parasites chemically. In some experiments they were chemically exsheathed. Leukocytes were obtained by venipuncture from chronically infected horses of different ages (2 days - 20 years) and parasite free foals. Larvae were pre-incubated or not with sera from parasite free foals or naturally infected animals, and were co-incubated during 3 days with leukocytes in different L3/cell ratios. Leukocytes from all horses, including a 2 days old foal and the 2 parasite free foals, consistently adhered to non-serum pre-treated larvae in about 1h. Leukocytes adhered equally to whole or exsheathed larvae. Pre-treatment of larvae with sera from infected or parasite-free animals significantly ($p < 0.01$) reduced adhesion time to about 30min. No differences in adhesion ($p > 0.1$) could be detected when different groups of cells or sera were compared. After 24-48h of co-incubation of cells and larvae, no lysis or reduction of motility of L3 could be detected. In 5/24 experiments with whole larvae and cells, unsheathed L3 could be detected; no unsheathed larvae could be seen in control cultures with no cells. Adhering cells were mainly eosinophils. Our results suggest that horse leukocyte adhesion to infecting larvae is non-specific and is non-specifically accelerated by serum, but does not clearly lead to parasite injury.

The predilection sites of louse populations on impala ewes (*Aepyceros melampus*) in the Northern Province, South Africa

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A study was conducted in the Northern Province, South Africa, to estimate external parasite numbers on specific sites on the body of impalas. Five lice species were collected from the hides of 45 impala ewes, *Aepyceros melampus* shot during July, October and February of 1994 and 1995. The aim was to record if different lice species exhibit any particular preferences for specific sites on the host's body and the role of seasonality. This study formed part of a larger study on the predilection sites of ticks on impala. Two destructive sampling techniques were used to remove all the lice from ten different sites. *Damalinia aepycerus* and *Damalinia elongata* were found predominantly on the body and tail. *Linognathus aepycerus* was widely distributed on most of the sites, and *Linognathus nevillei* which was recovered mostly from the hind leg, tail and front leg. An undescribed *Linognathus* sp. was also recovered, however in small numbers. No significant difference was found between the total number of lice recovered during the three sampling periods. There was however a correlation between the nymphal and the adult lice numbers on the different sampling sites.

The impact of environmental variation on the fitness of a land snail intermediate host and its larval nematodes

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Environments of five factors (temperature, humidity, nutrition, calcium, and parasitism) at three levels were used to produce life history data to elucidate the reproductive fitness variations for the land snail *Neohelix* (= *Triodopsis*) *albolabris*. First through third stage larvae (L₁ - L₃) of *Parelaphostrongylus tenuis* (Nematoda: Metastrongyloidea) commonly infect *N. albolabris* and were used for the parasite burden.

Snail responses to the experimental environments indicated significant variation in the number and quality of their eggs and offspring. Environmental factors also influenced nematode developmental rate and survivorship, thus the magnitude of the infective parasitic populations.

Environments favoring increases in adult snail activity allowed snails to accrue energy from foraging and to channel that energy into life history functions. Parasitic populations competed for nutrient-derived snail energy, with their effects being ameliorated by favorable host environmental tolerance and high nutritional levels. Larval development rates and survivorship were enhanced by low temperature and high nutrition host environments.

Life history functions in environments reducing the acquisition of nutrient-derived energy were characterized by delayed snail oviposition of large clutches with high hatching success, but also by high hatchling mortality and lower hatchling growth rates. The higher mortality and lower early growth rates imply limited energy resources were partitioned from adults into their eggs.

Development of a *Theileria parva* parasite of low pathogenicity as a vaccine

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A stock of *Theileria parva* isolated in the Lanet area of Kenya (NVRC stabilate 263) is characterised by its low pathogenicity and its ability to generate a broad spectrum of immunity against other, more virulent, parasite stocks. Before the Lanet stock can be developed as a possible "vaccine" against East Coast fever (ECF), however, it must satisfy a number of criteria. The mild responses to infection must be a result of low pathogenicity rather than a dose dependent response to the infective material injected. Reversion to virulence after cattle-tick-cattle passage must not occur. Daughter stabilates with the same characteristics as the mother stock must easily be produced.

Results from a series of investigations have shown that while the first two criteria can be met, production of a daughter stabilate has proved difficult due to the low number of piroplasms produced by infected calves and low infection rates in ticks feeding on the calves. In another trial, infected calves were injected with a corticosteroid to boost parasitaemias and enhance tick infection rates. A stabilate prepared from these ticks was titrated in calves. Results from the titration suggest that the corticosteroid modified the benign character of the mother stock and most of the calves developed severe reactions. Parasites were not detected in animals which received a low dose of infective material and they were not protected against a heterologous challenge.

MOLECULAR DELINEATION OF CYATHOSTOME SPECIES

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Cyathostomes are parasitic nematodes which inhabit the large intestines of the horse. Horses infected with these parasites may present with a variety of clinical syndromes; the most severe being larval cyathostomiasis, resulting in death in over 50 per cent of cases, despite intensive anthelmintic treatment and supportive care. Over 40 species of cyathostomes have been reported in the literature. Systematics of these species has been, until recently, solely based on morphological characteristics. It has only been possible to reliably differentiate the adult stages of these parasites to species level, at post-mortem. Recently, attention has been focused on the development of tests for antemortem species-identification, which could be applied at all stages of the parasite life cycle. DNA sequence data from the mitochondrial genome has been widely employed to resolve relationships among species and genera of parasitic helminths. In this study, single adult worm PCR was performed on a portion of the mitochondrial cytochrome oxidase I (COI) gene. From this, sequence data was generated, enabling us to determine the systematics and phylogeny of ten, morphologically identified cyathostome species, commonly isolated from our horse population. Species-specific differences have been determined from this sequence data, with potential for future application as an aid to differentiating some of the species present in cases of clinical disease.

Infection of cats with *Dirofilaria immitis* by simulated natural exposure to experimentally infected mosquitoes

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Simulated natural transmission of heartworm infection to young adult, laboratory-reared, domestic shorthair cats was accomplished by 2 methods. Infections in the cats were studied by testing periodically for heartworm microfilariae and antigen, and worm counts were made at necropsy. For both methods, each cat was anesthetized by IM injection of ketamine (15 mg/lb) plus acepromazine (0.15 mg/lb) for mosquito feeding. The number of infective third-stage larvae (L_3) received by each cat was estimated by subtracting the average number of L_3 per mosquito after feeding from the average number of L_3 per mosquito before feeding and multiplying this number by the number of mosquitoes that fed on the cat. For the first method, 18 (9M, 9F) cats were placed individually into a specially designed mosquito-proof cage. Fifty to 100 infected *Aedes aegypti* were released into the cage and allowed to feed for 45 minutes. The cats received an estimated range of 96 to 460 L_3 (avg. 214). One male cat had to be euthanized on Day 194 postinoculation (PI); one male cat died on Day 211 PI. The remaining cats were euthanized on Day 271 PI. Overall, 11 of 19 (61.1%) cats had heartworms (avg., 10.4/infective cat; range, 2-36). Seven of 11 (63.6%) infected cats had a few microfilariae on Days 195, 203, and/or 210. Six of 11 (54.5%) infected cats were positive on the antigen test (DiroCHEK®) at Day 168 and all 11 infected cats were positive on Day 195 and thereafter.

For the second method, 20 (10M, 10F) cats were infected individually by allowing caged infected *A. aegypti* to feed on a shaved area of the lateral aspect of the thigh. The estimated number of L_3 received by each cat ranged from 329 to 1194. Two male control cats died late in the study, with clinical signs resembling caval syndrome; one cat was dropped from the study. The remaining cats were euthanized on Day 178 PI. Thirteen of 19 (68.4%) control cats had heartworms (avg. 11.8/infective cat; range, 1-65). None tested positive for heartworm antigen until necropsy, when 2 were positive on the CITE® Semi-Quant test and 1 was positive on the Uni-Tec® CHW test.

Lymphocytes and immunity to nematodes in young lambs

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Identical twin lambs (8 pairs) were infected with 5000 infected larvae of *T. colubriformis* and either fed a high protein (150 % of the metabolizable protein needed for minimal growth) or low protein (100 % of MP) ration. One of each of the twin lambs was treated *in vivo* with an anti-WC1 antigen monoclonal antibody (CC15) which largely depleted the gamma-delta lymphocyte population. When the lambs were slaughtered 27 days after infection the established worm populations were not significantly affected by the depletion regime or by the dietary protein levels. The output of worm eggs was less in the high protein group ($p < 0.05$), but was not affected by the depletion. We conclude that the gamma-delta lymphocyte population has little or no effect on resistance in short term infections due to *T. colubriformis* in young lambs but that high protein diets inhibit the fecundity of establishing worm populations.



Immunity to nematodes during lactation in sheep

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Lactating and barren ewes (n=48) that had been previously trickle-infected, were compared with parasite-naïve ewes to assess immune responses following a challenge infection with *Trichostrongylus colubriformis* infective larvae (20,000). Random animals from within each treatment group were slaughtered 1, 7 or 21 days after challenge. All animals were individually fed in excess of ARC dietary energy requirements with a level of 15 % crude protein. There was a significantly greater ($p < 0.05$) establishment of parasites in lactating animals following challenge. The numbers of CD4+ cells present in the duodenal mucosa were higher in lactating animals ($p < 0.05$), 7 days postchallenge. However, *in vitro* proliferation of lymphocytes derived from the mesenteric lymph node of lactating ewes, to excretory/secretory L3 antigen was significantly depressed, at this same time ($p < 0.05$). Following trickle infection and at the time of challenge, there was lower levels of histamine in the duodenal mucus of lactating ewes ($p < 0.05$). The degree of larval migration inhibition (LMI) activity present in duodenal mucus was not significantly different between groups although a correlation of -0.5 existed between worm burden (log) and LMI among all treatment groups. We conclude that the lactational state confers a heightened susceptibility to *T. colubriformis* and the mechanism is likely to involve a change in the distribution and function of lymphocytes and globule leukocytes.

Epidemiology of nematode infections in cattle in Zambia, with special emphasis on the dry season and the impact of a single larvicidal treatment

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According to the findings of Chiejina and Fakae (1984), Kaufmann and Pfister (1990) and Ankers *et al.* (1992) pastures in subsahelian areas are free of infective trichostrongylid larvae during the dry season and reinfection of cattle, sheep and goats are thus negligible. Consequently, during the dry season, trichostrongyles survive in their host as hypometabolic adults or as arrested larvae.

The present experiment used monthly dung pads, monthly tracers and monthly egg counts to determine the situation in Lusaka Province, Zambia. It was aimed to determine in which form nematodes survive the dry season from March to October. Furthermore the impact on epidemiology and weight gain of a single larvicidal treatment with moxidectin during the dry season was determined in a controlled trial using Boran and Simmental cross first grazing season heifers. The animals were followed during one year.

The dung pads collected from various months and put in Baermann revealed a survival of as long as 165 days for all species found, *Cooperia* spp., *Haemonchus* and *Oesophagostomum*. With *Haemonchus* being apparently the most susceptible to adverse conditions of the dry season. Egg counts revealed on the other hand that there is no sharp drop in egg excretion at the start of the dry season but that the egg excretion only declines slowly, only to drop sharply in the middle of the next rain season. Together with the results of the tracers this indicates that at least part of the population of the parasites survives as active adults within the host. No evidence was found of any inhibited development in any of the species mentioned during the dry season. Finally the tracers showed clearly that infective larvae can survive on pasture for as long as 3.5 months after the end of the rainy season.

This study shows us a very different picture compared with the above mentioned studies, carried out in countries between the equator and the Sahara. It shows that larvae survive outside the host during the entire dry season, first as infective larvae on pasture, then as eggs/infective larvae in dung pads. Consequently a single anthelmintic treatment gave a reduction in egg counts during the dry season, but did not eliminate helminths from the treatment group. No difference in weight gain was observed at any time of the experiment.

Cloning and expression of an acetylcholinesterase from *Dictyocaulus viviparus*

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Many nematode species secrete acetylcholinesterases (AChEs). These enzymes have been proposed a role in immune evasion via the breakdown of host acetylcholine (ACh). In addition to its neurological function, ACh has been shown to stimulate activity of various types of immune effector cell. Adult stages of the bovine lungworm, *Dictyocaulus viviparus*, release profuse quantities of AChEs *in vitro*. These enzymes are targets for circulating antibodies in calves rendered immune by infection, suggesting that they are released whilst the parasites are resident in the host lungs. *D. viviparus* fractions, enriched for AChEs, have been shown to be protective in guinea pigs. To study the role of these enzymes further, a gene encoding *D. viviparus* AChE is being cloned and the recombinant enzyme expressed.

A 326 base pair (bp) fragment was generated by reverse transcription-polymerase chain reaction (RT-PCR) on RNA isolated from adult *D. viviparus*. This fragment was amplified using degenerate primers designed from published sequence of the *Caenorhabditis elegans* AChE gene, *ace-1*. This fragment showed 58.4% identity to *ace-1* and more than 60% identity to cholinesterases of other species. In an attempt to yield a full length clone, the 326 bp fragment was used to screen a *D. viviparus* adult cDNA expression library. From this, a partial length (1.2Kbp) cDNA was identified. Sequencing showed that this clone had 55 to 58% similarity to other cholinesterase genes. From the 1.2 Kbp clone, an internal fragment was generated by PCR and used to rescreen the library at higher stringency. This yielded a putative full length AChE-encoding cDNA which was sequenced and subsequently subcloned into a suitable expression vector.

Persistent efficacy of moxidectin 1 % injectable and 0.5 % pour-on against natural *Psoroptes ovis* infestation in cattle

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The 28 days persistent efficacy of moxidectin 1 % injectable and 0.5 % pour-on was evaluated under field conditions in Belgian White and Blue heifers naturally challenged with *Psoroptes ovis*.

The entire trial was conducted on a private farm with a history of recurrent *P. ovis* mange. Based on age, body weight, affected body surface (clinical score) and mite count three equivalent groups of 15 females each were formed. On Day 0 (D0), groups A and C were treated with a single dose of moxidectin injectable (0.2mg/kg b.w.) and pour-on (0.5mg/kg b.w.), respectively. Group B was treated with flumethrin pour-on (a non remanent pyrethroid drug) twice on D0 and D10. All 45 animals were kept in the same pen and exposed from D21 to D28 to a natural challenge by introducing 8 heifers naturally infested with *P. ovis*. At the end of the challenge, the 3 treatment groups were separated and checked once a week until D66. Clinical scores and mite counts were recorded.

The clinical scores decreased in all 3 groups until D49; on D56 and D66, they remained quite stable in groups A and C but significantly increased in group B. In groups A and C, no mites were found at any time after treatment and challenge whereas in group C, 7 %, 11 %, 14 % and 62 % of the examined animals harboured live mites on D42, D49, D56 and D66, respectively.

It is concluded that under controlled conditions both formulations of moxidectin were able to protect cattle against reinfestation for a minimum of 28 days after treatment.



Evidence of transmission of *Theileria parva* by *Rhipicephalus appendiculatus* on cattle under tick control by handspray acaricide application

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Theileriosis, caused by *Theileria parva* and transmitted by the tick *Rhipicephalus appendiculatus*, is a major economic threat to the cattle industry in Eastern and Southern Africa. In the traditionally sector, the most common control method is by the application of acaricide.

In May 1992, an outbreak of East Coast Fever (ECF) was observed in an existing experimental herd in the Central Province of Zambia. The following study compared the incidence of ECF under natural challenge, observed in two different herds: one under fortnightly acaricide application, and another not subjected to any tick control. All the animals were checked daily. Lymph node biopsies and blood smears were taken from all animals with a body temperature above 39.5°C. Following diagnosis of the first cases of ECF the treated group was sprayed weekly to increase protection and avoid further losses.

Despite a reduction of risk by 79.5 %, the intensive tick control failed to completely protect the cattle, as an incidence of 0.0009 cases per day was observed throughout the outbreak period of 90 days. The acute nature of the disease r

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Cestode parasites of fish and piscivorous birds from the Northern Province of South Africa.

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A survey of the gastrointestinal helminths of the Phalacrocoracidae and the Anhingidae in the Northern Province revealed, amongst others, six species of cestodes, viz. *Ligula intestinalis*, Linnaeus, 1758; *Paradilepis scolecina*, (Rudolphi, 1819); *P. delachauxi*, (Fuhrmann, 1909); *Amirhalingamia macracantha*, (Joyeaux and Baer, 1935) Bray, 1974; *Echinorhychotaenia tritesticulata*, Fuhrmann, 1909 and *Hymenolepis cormoranti*, Ortlepp, 1938.

The role of piscivorous birds in the life cycles of cestodes of South African freshwater fish species has, at yet, received little or no attention at all. This study established certain fish-avian parasitic linkages. The reliability of morphological features used to link larval and adult forms of the procured cestodes are assessed and discussed.

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The helminth parasites of dogs and their socio-economic impact in resource-limited communities in South Africa

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The aim of the study is to determine the socio-economic role of dogs in resource-limited communities, to define their health status and extent of veterinary care, to investigate the occurrence of parasites within these communities (with special reference to helminth parasites) and to assess the zoonotic helminth potential in order to develop appropriate means of internal parasite control. Information on the socio-economics of these communities will be gathered by means of a questionnaire. Collection of samples will include the following: faecal samples (fresh) collected per rectum for demonstration of nematode eggs, purged faecal samples for demonstration of tapeworm segments, blood films (smears) in order to detect microfilariæ and protozoa, heparinised and/or EDTA blood samples for acid phosphatase staining of microfilariæ, and adhesive tape swabs of perianal skin for demonstration of tapeworm eggs. Dogs will also be examined clinically in order to determine health status, body condition and collect external parasites if present.

Seroprevalence studies involving *Toxocara* spp. alone in humans that were published in 1988, were found to be as high as 80% in children and 47.5% in adults in Venezuela. South African figures are outdated, but the latest (1979) indicated prevalence of 31% for *T. canis*, 11.9% for *Ancylostoma caninum*, and 1.5% for *Echinococcus* spp. in the Pretoria area. This is disturbing, as 80% of positively diagnosed cases occurred in urban areas. Many helminth parasite species found in dogs can seriously affect humans as their paratenic hosts (most important), intermediate hosts, potential hosts, or opportunistic hosts. Detrimental effects of these parasites are in some adult cases inapparent, but more pronounced in children, and may range among the following: asthma, abdominal discomfort, cholic, seizures, epileptic fits and ocular damage. Determining the impact on health and socio-economics is vital in order to formulate cost effective and appropriate recommendations for control of these zoonoses in order to promote community health and well-being as a whole.

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Enhanced activity of moxidectin and ivermectin against selected strains of *Haemonchus contortus* in jirds by co-administration of the MDR-reversing agent verapamil

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The effect of moxidectin (MOX) and ivermectin (IVM) was examined in jirds in association with verapamil (VRP), a calcium channel blocker with multidrug resistance (MDR) properties, on strains of the ruminant parasite *Haemonchus contortus* selected with MOX, or unselected. The objective of the present study was to determine whether a MDR reversing agent can be used to restore the efficacy of macrocyclic lactones against resistant parasites. MOX-selected strain of *H. contortus* demonstrated a 3 and 3.1 (LD50) fold increase in resistance to MOX, and IVM, respectively compared to the unselected strain. MOX (0.017 mg/kg) alone and MOX in combination with VRP (20 mg/kg) had significantly different cure rates ($P=0.012$) of 70% and 96%, respectively. IVM (0.028 mg/kg) alone and IVM in combination with VRP had significant different cure rates ($P=0.02$) of 80% and 93%. VRP given alone had no antiparasitic effect. VRP is purported to act by competing for the drug binding site on the membrane transport protein P-glycoprotein (P-gp) in MDR cancer cells. P-gp has recently been demonstrated to be over expressed in resistant strains of *H. contortus*. These data suggest that VRP may also block P-gp mediated efflux effect of macrocyclic lactones in drug selected *H. contortus*. This new treatment strategy of parasite control using an anthelmintic/MDR modulator association, may be useful for restoring the efficacy of macrocyclic lactone anthelmintics against strains of parasites with reduced susceptibility to these anthelmintics. /



Activity of moxidectin and ivermectin against moxidectin selected and unselected strains of *Haemonchus contortus* in jirds

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Ivermectin (IVM) resistance has been reported in many parts of the world. In the field, IVM-resistant strains have been found to be susceptible to use level moxidectin (MOX). However under experimental conditions, IVM-resistance has been found to affect sensitivity to MOX. Little information is available on the effects of intensive MOX selection on sensitivity to MOX and IVM. After twelve generations of selection with varying concentrations of MOX in sheep, the MOX-selected strain of *Haemonchus contortus* (MOF12) demonstrated a 5.3 fold decrease in sensitivity to MOX compared with the parent unselected strain (PF12) which was passages at the same time for twelve generations. In this study, the effects of MOX and IVM on the MOX selected strain, after fourteen generations of selection (MOF14), and on the unselected strain (PF14) of the ruminant parasite *H. contortus* were examined in jirds (*Meriones unguiculatus*). Based on the recovery of early adult worms and L₄ larvae from the stomach of jirds, MOX and IVM, both at a dose rate of 0.025mg/kg, were 96 % and 55 % effective against the PF14 strain, respectively. MOX at 0.025mg/kg was 61 % effective when used against the MOF14 strain and IVM at the same dose rate was 34 % effective against the MOF14 strain. The results indicate that MOX was more efficacious than IVM when jirds were treated with subtherapeutic doses of both endectocides. The jird model can be used effectively to investigate the response of anthelmintic sensitive and resistant strain of *H. contortus* to chemotherapy.

Evolution of lymphocyte subsets in *Fasciola hepatica* infected sheep

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Peripheral blood lymphocytes (PBL) subsets were characterized weekly in *F. hepatica* infected sheep during six weeks post-infection (WPI) using flow cytometry. After *in vitro* stimulation with *F. hepatica* excretory-secretory products (FhESP), these lymphocytes were analysed using flow cytometry and IFN γ quantification. The total number of PBL was not changed during the experiment, but the different lymphocyte subpopulations rates were modified between WPI1 and WPI3 with an increase of B lymphocytes and a decrease of OvCD5+, OvCD4+, OvCD8+ lymphocytes. No variation in $\gamma\delta$ TCR+ lymphocytes number was revealed. OvCD4+ lymphocytes decreased in peripheral blood whereas they increased in both hepatic parenchyma and hepatic portal tracts as previously described. In contrary, discordance between general and local responses for OvCD8+ lymphocytes should be investigated. FhESP specific lymphocyte proliferation was precocious and transient (WPI2 to WPI5), as previously observed. No differential stimulation of lymphocyte subsets was observed using flow cytometry but IFN γ secretion by stimulated lymphocytes increased in WPI1 and WPI2. Further investigation will be necessary to estimate the role of lymphocyte subpopulations in immune regulation of fasciolosis.

Stephanofilariid dermatitis (humpsores) in working cattle in Bangladesh

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Dermatitis in cattle with different species of the genus *Stephanofilaria* (Spirurida: Setariidae) more or less occurs in all the continents of the World. The disease entity has been referred to various names, depending upon the parasite species, geographic locations and also the lesions present on the host's body. Humpsores, caused by *S. assamensis*, a typical skin lesion on the hump of Zebu cattle is rampant in Bangladesh, India and parts of the former Soviet Union. This paper deals with humpsores in working cattle in Bangladesh.

Skin scrapings examination of 246 bullocks and cows, aged between 7 to 9 years, revealed *S. assamensis* infestation in 98(39.8%) cases. Although hump has been said to be the site of infestation, but about 60% infestations were found on the neck, 35% on the hump and 5% around the base of the ears and/or horns. The size of the lesions on the hump and neck varied from 5 cm to 13 cm, and were chronic granulomatous to ulcerative in nature. Blood and serous exudate oozed out as the animals frequently rubbed their affected parts of the body with the pillars, trees and the like. In some protracted cases suppuration was not uncommon. These attracted flies, mostly *Musca conducens*, the main vector of *S. assamensis*.

Most of the affected animals refused yoke while ploughing and/or carrying the cart and thus hampering agriculture. A daily loss of Taka 100 (approx. US\$3) was estimated due to inability of a bullock to work. This is a great concern to the peasant community. An ointment composed of 10% Neguvon (trichlorphon), 5% sulphaniamide and 5% Zinc oxide applied topically twice daily on the affected area was found effective. The amount of ointment (30 grams) needed for an animal costs Taka 10 (approx. US\$0.3) which was within the reach of the common farmers.

Response of Dorper and Red Maasai lambs to primary, secondary and tertiary infections with *Haemonchus contortus*

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The abomasal nematode *Haemonchus contortus* is a major constraint on sheep production in tropical and subtropical areas and breed resistance is thought to be a potential that can be exploited to control the parasite. In this regard, a series of experiments were conducted with a view to confirming the previously reported superior resistance of the Red Maasai breed to haemonchosis. In the present experiment, Red Maasai and Dorper lambs with apparently little prior exposure to *Haemonchus* parasites were infected. No breed differences in resistance in terms of either faecal egg counts or total worm burdens were observed. Faecal egg counts decreased with each infection probably because of increasing resistance associated with prior exposure and increasing age. The lack of any significant breed difference was also reflected in the packed cell volumes (PCV), eosinophil and serum IgG results. Although eosinophil responses appeared to increase with each infection in both breeds, the marked eosinophilia during and after.

Ectoparasites, endoparasites and haemoparasites in backyard flocks in rural Zimbabwe

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A survey on poultry production is being carried out in backyard flocks in the rural areas of Zimbabwe with emphasis on the impact of ectoparasites, endoparasites and haemoparasites on poultry production. Preliminary studies in Chiweshe Communal Area have shown high prevalence of ectoparasites (mainly lice) and endoparasites (mainly nematodes and cestodes) and low prevalence of haemoparasites out of a total of 50 chickens slaughtered. Among the species of cestodes identified so far two of the species (*Cotugnia diagnopora* and *Railletina* (*Pironella*) spp.) constitutes new records in Zimbabwe and of the species of nematodes one species (*Skrjabinocerca* spp.) is a new record. Nine species of ectoparasites have been recorded (3 species of lice, 4 species of mites, 1 species of ticks and 1 species of fleas). To assess the pathological changes attributable to the presence of these parasites, histopathological examination of the organs affected were carried out.

Efficacy of ivermectin delivered from a sustained-release (IVM SR) bolus against gastrointestinal (GIT) nematodes in field grazing calves in Nyandarua district, Kenya

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The efficacy of ivermectin sustained release (IVM-SR) bolus in reducing GIT nematodiosis in field grazing calves was evaluated over a period of 140 days in 12 steers of various European breeds aged approximately 8-10 months and ranging in weight from 150-175 kg. They were allocated to two groups of 6 steers each by restricted randomization based on their liveweight. Each of the treatment group received an IVM-SR bolus designed to deliver 12 mg of the ivermectin/day for 135 days. The other group was designated as non-medicated controls.

The groups were placed on adjacent 3-acre paddocks obtained by subdividing of a 6-acre permanent pasture which had previously been grazed by young untreated cattle to exposing experimental steers to a similar risk of infection from GIT nematodes.

All animals were weighed and rectal faecal samples including jugular blood samples for serum extraction were collected from each animal, beginning on Day 0. Herbage larval counts, live weight gain and serum pepsinogen levels were monitored at regular intervals until termination (Day 140), when the steers were removed from pasture, housed in isolation for three weeks then necropsied for recovery of GIT nematodes. Three sets of parasite naïve tracer male calves were utilized to evaluate the initial, interim and final stages of the experiment.

The use of IVM SR boluses resulted in the reduction in EPG of trichostrongyles and number of GIT nematodes in the treated principal animals, as well as daily liveweight gain advantage over the 140 day period. Similarly there was a reduction in pasture larval nematode contamination of pastures grazed by the treated animals, as indicated by the parasite burden in tracer calves and pasture larval counts. The control steers required occasional salvage treatments over the trial period.

Correlation of specific antibody titre and avidity with protection in cattle immunised against *Fasciola hepatica*

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Two groups of cattle were immunised with a combination of fluke derived antigens (cathepsin L2 and hemoglobin) in oil adjuvant. Subsequently, these animals were challenged orally with 500 viable *Fasciola hepatica* metacercariae and were examined for fluke specific serum antibody isotypes IgG1 and IgG2 by ELISA. The avidity of each isotype for fluke antigen was established using the Potassium thiocyanate elution method, and the fluke burdens were assessed at post mortem.

Cattle produce specific serum antibody mainly of the IgG1 isotype in response to challenge. In contrast, vaccination induced a specific antibody response involving IgG2, as well as IgG1. Immunised cattle given their primary immunisation with the antigens formulated in Freund's complete adjuvant had fluke burdens 72.4% lower than those of the challenged controls. When the primary immunisation was given in Freund's incomplete adjuvant the specific antibody response, while still involving IgG1 and IgG2, was of lower magnitude, and no significant reduction in fluke burden was observed following challenge.

When the immune responses of immunised and challenged control animals was compared, regression analysis indicated a positive correlation between fluke specific IgG1 levels and fluke burden. Whilst a strong specific IgG2 antibody response was associated with low fluke burdens. These IgG2 antibodies were shown to be significantly higher in avidity than specific serum IgG1 antibodies.

These results provide evidence of the non-protective nature of specific immune responses in cattle following *F. hepatica* infection, and demonstrates that vaccination can induce a qualitatively different, protective response.

Extra-intestinal migration of *Ascaris suum* in the pig - an experimental study.

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The extra-intestinal migration of *Ascaris suum* in pigs is believed by most researchers to follow the so-called hepato-tracheal route. However, in the older literature some investigators have suggested that the larvae may follow alternative routes, i.e. they may enter directly into the peritoneal cavity and from there reach the liver and lungs, or they may follow the intestinal lymphatic system and via the thoracic lymphatic duct reach the blood circulation and hence the lungs. Recent observations in our laboratory also question whether the hepato-tracheal migration is the only option.

In an attempt to elucidate the potential routes of migration we surgically injected artificially hatched larvae into caecal veins, caecal lymph nodes and peritoneal cavity, around the large intestine of parasite-naïve pigs. We recently demonstrated that the large intestine is the site where *A. suum* larvae normally penetrate into the gut wall (Murrell et al., J. Parasit. in press 1997). For comparison we perorally inoculated parasite-naïve pigs with infective eggs of the same batch. On day 4 all categories of pigs showed white spots and had larvae in their livers, yet the peroral and intravenous pigs had considerably higher numbers than the other pigs. On day 13 we recovered larvae from the small intestine of all categories of pigs, the numbers in the perorally inoculated pigs exceeding those of the others which were largely comparable. The present results may suggest a higher diversity of *A. suum* migration than normally anticipated.

Characterization of *Anaplasma* isolates from eland (*Taurotragus oryx*):

Pathogenicity in cattle and sheep and DNA profiles analysis

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Two eland *Anaplasma* isolates, Anap_{E1}, from Kenya and Anap_{E2}, from South Africa were characterized. Their characterization was based on their pathogenicity to intact and splenectomized cattle and sheep and also their DNA profiles. Their DNA profiles were analyzed and compared to *Anaplasma marginale*, *A. ovis* and *A. centrale*, after endonuclease restrictions and probing with *Anaplasma* DNA probes, AC 5-12 and AC-1. The results of the pathogenicity trials showed that Anap_{E1} was similar to *A. ovis* and Anap_{E2} similar to *A. marginale*. On DNA profiles, Anap_{E1} was close to *A. ovis*, with only differences that often occur in same *Anaplasma* species isolated from different locations. On the other hand, Anap_{E2} resembled one of the *A. marginale* isolates known to occur in South Africa. The DNA profiles correlated well with the pathogenicity results. It is concluded that elands are carriers of both *A. marginale* and *A. ovis* parasites and may therefore be important reservoirs that need attention in epidemiology of anaplasmosis.

Research of Effectiveness of Two Various Systems of Pig Liquid Manure treatment from the Microbiological and Parasitological Point of View.

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The objective of this work was to study the influence of two systems of pigs liquid manure treatment - long term storage under aerobic condition and anaerobic fermentation on microbial (processing and hygienic - epizootologic indicators) and parasitological population.

After the six weeks of storage of liquid manure was proved the great decrease of mesophilic and psychrophilic microorganisms. From the point of view hygienic epizootologic indicators: any significant changes of coliform microorganisms were observed, fecal coliform microorganisms were not detected in winter conditions from the eleventh week. The amount of moulds we were not able to detect after nine weeks.

During Anaerobic fermentation of pig liquid manure the number of mesophilic and psychrophilic microorganisms, as well as coliforms and faecal coliforms decreased by 2-3 log units. Total devitalization coliforms and faecal coliforms in most samples was reached during storage in collected tanks.

Oocysts of genus *Eimeria*, *Isospora*, *Cryptosporidium* and cysts of *Giardia* and *Balantidium* were identified together with eggs of *A. suum*, *Trichuris* spp. and *Hymenolepis* spp. in samples by direct methods. In the liquid manurestuff for spreading to fields the total devitalization of parasitological eggs was founded.

Seasonal transmission of *Fasciola gigantica* infections and the possibility of a strategic anthelmintic control in an endemic area of Central Kenya

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A study to investigate the seasonality of infections with *Fasciola gigantica* was carried out in Mathira Division of Nyeri District, Kenya from June 1995 to August 1996. Young Dorper lambs were used as tracer animals every month and they grazed alongside farmers' flocks. Adult ewes were also bought from the flocks in the area. These two groups were necropsied after a three and six week housing under worm-free conditions and all flukes in the livers recovered and counted. The tracer lambs and permanent sheep (adult ewes) were infected with *F. gigantica* most of the months. Peaks of infection in both tracer lambs and permanent sheep appeared in October 1995 and February 1996.

It is recommended that one main strategic treatment with triclabendazole instituted in October can significantly reduce the production losses associated with fasciolosis. Clinical cases of fasciolosis can be treated individually when they occur during the other times of the year.

Efficacy of liposomal albendazole against experimental *Trichinella spiralis* infection in mice

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Efficacy of albendazole entrapped in neutral and negative-charged multilamellar liposomes with high and low level of phospholipids was evaluated against experimental *Trichinella spiralis* infection in mice at dose level ranged from 50 to 200 mg/kg of body weight by dermal application. Mice-males with body weight of 25-30 g were infected with 150 *Trichinella* larvae per one animal. On 35 day of infection liposomal albendazole was applied at hairless skin area (2 x 2 cm²) on back during 7 day period. The day dose of liposomal albendazole was divided in half and applied twice with 4-hour interval. 7 animals were in each test and control groups.

The results of treatment were assessed 28 days after the last dosage of liposomal compositions by trichinelloscopy method and digestion technique in artificial gastric juice.

Albendazole entrapped in neutral liposomes with low level of phospholipids applied topically at dose level of 200, 150 and 75 mg/kg of body weight was 95,4%, 100,0% and 84,2% effective respectively. Negative-charged liposomes with albendazole at dose level of 75 mg/kg of body weight administered by dermal application showed efficacy of 84,4%.

Liposomal albendazole with high level of phospholipids at dose level of 50 and 75 mg/kg of body weight demonstrated efficacy close to 100% (99,1% and 98,7% respectively). The authors considered liposomal albendazole to be a highly effective composition against muscular trichinellosis.



Epidemiology of tick-borne diseases in the various cattle of production system of Eastern and Central region of Uganda

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No abstract received

Attempts to optimize ivermectin treatment of reindeer

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The semi-domesticated reindeer harbour a variety of different parasites. Most of the important species belong to arthropods and nematodes, and are easily killed with antiparasitics. Oestrid fly larvae, warbles (*Hypoderma tarandi*) and throat bots (*Cephenemyia trompe*), are the most evident parasites, but gastrointestinal nematodes are also highly prevalent. The high efficacy against oestrid larvae has made ivermectin very commonly used in the reindeer husbandry. In Finland, over 80 % of the stock are treated once yearly, during autumn or winter. The pronounced seasonality of the reindeer and its parasites has helped the success of such treatment; the first possibility of reinfection is often during the next summer.

The high efficacy of ivermectin against the most evident parasites has led to the herdsmen developing new treatment regimes. Instead of calling an expensive veterinarian to inject ivermectin, many herders have started to use ivermectin paste developed for horses. Moreover, because this paste is relatively expensive, the most innovative reindeer owners have reduced the dose from the original 200 µg/kg down to 50, still retaining high efficacy against oestrid larvae.

To elucidate the potential problems, we started experimenting with ivermectin in the Kaamanen Experimental Reindeer Herd. We compared the antiparasitic efficacy of subcutaneously, orally and topically administered ivermectin. While all the treatments were highly effective against warbles and throat bots, gastrointestinal nematode egg production in the spring was retarded only after subcutaneous administration. The explanation is in plasma ivermectin concentrations; the low and short-lasting ivermectin concentrations in orally treated animals indicate that ivermectin is poorly absorbed from the gastrointestinal tract of reindeer. The probable reason is that ivermectin is adsorbed to ingesta, much of it going to the dung. So, we consider even the 200 µg/kg oral dose to lead to underdosage regarding gastrointestinal nematodes.

No difference was seen in the antiparasitic efficacy or production efficacy between hinds treated in September, December, or February, respectively.

Based on the current knowledge, we recommend reindeer herders the use of ivermectin only subcutaneously to ensure the drug reaches its targets, and at any time during the autumn or winter (when the flock is collected). As the work continues, these recommendations are subject to change.

Efficacy and persistent effect of ivermectin controlled-release capsule and ivermectin 1 % injection against *Melophagus ovinus*

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The therapeutic and prophylactic efficacy of ivermectin administered by controlled-release capsule (CRC) or by injection against *Melophagus ovinus* was evaluated in sheep in Patagonia, Argentina. Thirty Merino/Corriedale cross sheep, aged 3-4 years and weighing 34-64 kg were used. The sheep were formed into blocks of three animals from a ranking on ked counts within sex. Within each block, one animal was randomly allocated to each of three treatment groups. One group acted as untreated controls. Sheep of the other two groups received ivermectin by injection at 200 mcg/kg administered subcutaneously on Days 0 and 21 or by CRC formulated to deliver ivermectin at a minimum dose of 20 mcg/kg/day for 100 days administered once on Day 0. The sheep were housed in group pens with five animals from a single treatment group in each pen. Keds were counted on the right side of each animal on Days -2, 21, 40/41, 49, 56, 70, 77, 91 and 105. At Day 49, no keds were found on the injection-treated sheep or on 9 of 10 CRC-treated sheep. No keds were found on any of the ivermectin-treated sheep on Days 56 and 70. All 10 control animals were positive on each occasion (mean > 70). After the Day 70 count, the treated and control animals were penned together by replicate until Day 77, when 20 keds were manually transferred from control animals to each ivermectin-treated sheep and the groups were moved to separate pens again. The ivermectin injection treatment was not expected to provide any efficacy by the Day 70 to 77 challenge period and, in this group, all 10 animals were positive for keds on Days 91 and 105 (mean 4.9 and 7.6, respectively). Only low numbers of keds were found on some CRC-treated animals at the same count times (mean 1.3 and 0.9, respectively). The results demonstrate that ivermectin delivered by CRC or as two injections 21 days apart provides 100 % efficacy against *M. ovinus* infestation in sheep. In addition, the CRC reduces the level of infestation resulting from challenge with *M. ovinus*.

Productivity field trial using Ivermectin 1% injection in programs for mange control of sheep in Patagonia, Argentina.

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Psoroptes ovis is responsible for the most important sheep disease in Patagonia. The objective of this study was to measure comparatively the effect of control, on production and quality of the wool in *P. ovis* affected sheep, using traditional dipping and parenteral treatment. Ninety two Merino lambs were each infested in autumn, by placing 15 to 20 mites on the skin and were allocated in three similar paddocks. Group 1 (G1) (control) was not treated, Group 2 (G2) was given two dips with diazinon after obvious clinical signs of mange were observed and according to weather conditions (traditional management). Group 3 (G3) was dosed, as soon as mange was diagnosed, with ivermectin (injectable solution at 200 mcg/kg) twice, seven days apart. The result of the treatment in G2 and G3 was a complete control of *P. ovis*. In G1, 4 sheep died exhausted. The evolution of mean body weight between groups was statistically different ($P < 0.05$). At shearing, all sheep from G1 showed depilation, and wool characteristics such as wool weight, fineness, staple length, staple strength, suint and yield were statistically different ($P < 0.05$) between groups, G3 showing the best performance. A cost/benefit analysis also showed a better performance for G3. This group has a ratio of 42 % better than G2. The findings sustain that parenteral treatment with ivermectin as soon as the mange is diagnosed is highly recommended in patagonian affected flocks.

First confirmed case of cerebrospinal setariosis in goat in Taiwan

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Setaria digitata is known to parasitize in the peritoneal cavity of cattle without any apparent clinical symptom in Taiwan. Despite that cerebrospinal setariosis in goat has been suspected in Taiwan, no worm has ever been discovered.

We present in this paper the discovery of a larva of *S. digitata* from the brain of a paralyzed and epileptic goat in Taitung, eastern Taiwan. The goat was from a farm which reared about 350 meat and dairy goats. In the summer (July and August) of 1994 and 1995, four and seven goats in that farm showed hind limb paralysis, respectively. In July 1996, four more goats showed the same clinical symptoms. Necropsy was performed on one of the goats and a female larva of *S. digitata*, measuring 2.15cm was found in the cerebral aqueduct. Neutrophils were observed in the spinal fluid smears and no aerobic nor anaerobic bacteria was isolated from the central nervous system. Serum creatine kinase (CPK) of the goat at necropsy was elevated several hundred-fold of the normal value. Analysis of the CPK isoenzyme showed that it consist of 100:0:0% of MM:BB:MB. No significant cellular infiltration was observed in the histopathological section of the brain. This is the first confirmed case of cerebrospinal setariosis in goat in Taiwan.

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Recommended methodology for efficacy trials of anthelmintics in dogs and cats.

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Modern anthelmintics for dogs and cats have spectra of activity which include a wide range of both cestodes and nematodes. This makes artificial infestation very difficult.

A good source of naturally infested dogs and cats is animal welfare organisations, where animals that are due to be euthanased can be randomly divided into treated and control groups. After euthanasia the gastro-intestinal tracts can be removed from the trial animals and taken to the laboratory for total worm counts and species identification.

Cestode infestation is best diagnosed by the presence of segments in the faeces. Nematode infestation is best diagnosed by the presence of eggs in the faeces, with the help of a three-part gauze filter which filters out nematode eggs into one compartment, cestode and trematode eggs into another, and the larger portions of faecal detritus into a third.

It is important to have a certain number of helminths of each species in the control group. This type of trial can be continued (in different areas if necessary) until the required number of each helminth species has been included.

Colonization of cattle and sheep feces by nematophagous fungi in Brazil

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Ruminant feces are a potential environment for nematophagous fungi to colonize and act on the free-living stages of trichostrongylid nematodes. In order to identify the species which naturally colonize cattle and sheep feces and the speed at which the colonization occurs, feces of these animals were spread on *Brachiaria decumbens* pastures in the Mata Region of Minas Gerais State (MG). Cattle pats were spread monthly from April, 1995 to April, 1996, while sheep feces were deposited on the months of July and October, 1995 and January and April, 1996. Samples were collected at 3, 7 and 14 days after fecal deposition for culture in water-agar containing nematodes as baits. Inoculated plates were examined for three weeks. Isolated fungi were further cultured in corn-meal agar plates for additional studies, classification and characterization. From the 390 cattle pats deposited in the whole period, 293 fungi were isolated: 28 in April, 1995, 19 in May, 12 in June, 11 in July, 10 in August, 20 in September, 24 in October, 18 in November, 13 in December, 26 in January, 1996, 35 in February, 54 in March and 23 in April. On day 3, 7 and 14 the percentages of isolated fungi on bovine feces were 26.3 %, 29.2 % and 44.5 %, respectively. A total of 123 fungi were isolated in the 120 sheep fecal sample deposited on pastures: 22 in July, 39 in October, 41 in January and 21 in April. Most fungi were isolated at the third day after deposition (35.6 %). Twenty eight species of fungi were classified in the isolates: 22 predators and six endoparasites. Among the predators there was a predominance of fungi which produce adhesive nets, which were able to colonize in the dry as well as in the wet season. *Arthroboyrys oligospora* predominated in the bovine feces while *A. oligospora* and *Monacrosporium eudermatum* predominated among the sheep isolates. *Myzocyrtium* sp. and *Harposporium anguillulae* were the species which predominated among the endoparasites. Cattle and sheep feces colonization by nematophagous fungi in the Mata Region of MG was fast, diversified and influenced by climatic conditions. Additional studies aimed at the characterization of the nematophagous activity of the isolates against free-living stages of trichostrongylid nematodes are being conducted.

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Trends in commercial research on parasiticides.

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Considerable advances have been made in the methodology of field research on the safety and efficacy of parasiticides over the past twenty years, both in South Africa and internationally.

In the nineteen seventies the registration authorities began to insist on proper controlled efficacy trials for all parasiticides, even those of a naturopathic or homeopathic type. In the early nineteen eighties the trend was towards including sufficient replicates to make the results statistically significant.

In the mid-eighties the trend was towards testing the remedy in different environments and climatic conditions. In the early nineties trials on naturally-infested animals became more difficult because the modern remedies already in use were so effective that it was often difficult to obtain sufficient challenge for the test remedy.

Now in the mid-nineties the trend is towards applying good laboratory practice (GLP) standards. This has the advantage of making trial results more reliable and comparable, and in the future it will also hopefully allow harmonisation of registration requirements between countries. This should help to offset the increase in trial costs.



Comparison of resistance of Suffolk and Belgian Voskop sheep to *Haemonchus contortus*

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The objective of the study was to compare the response of Suffolk and Voskop (a native Belgian sheep) to experimental infection with *Haemonchus contortus*. Two sets of experiments were carried out. In experiment one, 18-24 months old females were orally infected with 250 infective larvae (L_3) of *H. contortus* per kg of body weight, treated with fenbendazole at day 35 and challenged with 20,600 L_3 at day 42. An uninfected control and a challenge control group was also used. In experiment two, 8-9 months old lambs of both sexes were given trickle infection of 7 doses of 1000 L_3 every 3 days for 3 weeks, treated with levamisole on day 50 and challenged with 9000 L_3 on day 57. Uninfected and challenge control groups were also used. Weekly faecal egg counts and blood analysis were done till day 77 in experiment 1 and till day 99 in experiment 2. In experiment two, 12 infected animals were necropsied on day 99 for worm recovery. In experiment 1, there was no significant difference ($P=0.064$) between the egg counts of two breeds although the Suffolk had consistently higher counts than the Voskop. In experiment 2, during trickle infection, Suffolk had significantly higher egg counts than Voskop ($P<0.01$). However, after challenge there was no significant difference between the egg counts of two breeds although the counts of Voskop were 27% lower than that of Suffolk. Worm counts in challenged lambs of Suffolk were 2.4 times higher than those of Voskop, but the difference was not statistically significant. In both experiments there were large individual variations in egg counts as well as in worm counts. It is concluded that Voskop is more resistant than Suffolk, trickle infection is a better way of detecting breed differences and large individual variations may be exploited for genetic selection of animals for worm resistance.

Evaluation of diagnostic techniques for *Trypanosoma evansi* infection in dromedary camel

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Two hundred and eight camels, symptomatically suspected of surra, living in *Trypanosoma evansi* endemic areas of the Thar Desert of Rajasthan State of India, were selected. The efficacy of various diagnostic tests viz., the parasitological tests (wet blood film - WBF, stained thick blood film), chemical test (mercuric chloride), biological test (mouse subinoculation - MSL), and immunodiagnostic tests based on antibody detection (double immunodiffusion test - DID, card agglutination test - CATT), antigen detection (double antibody sandwich enzyme immunoassay - Ag-ELISA) was comparatively evaluated. The detection of anti-trypanosome antibodies, and trypanosomal antigens was correlated with parasitological diagnosis. Of the tested camels, 99 were found infected using the WBF of which nine gave false negative results with mercuric chloride test. The efficacy of MSI was 87.03 percent, while mercuric chloride test was 60.18 percent efficient. The diagnostic efficacy of CATT (72.22 percent) was found to be much better than DID (28.70 percent). Ag-ELISA was 86.11 percent efficient in detecting trypanosomal antigens. A good correlation was found between the positive results obtained by wet blood film CATT and Ag-ELISA. It was inferred that CATT can be used to study the seroprevalence of *T. evansi* with great ease, however, trypanosome antigen detection may give a more accurate idea of the prevalence of *T. evansi* in an endemic area.

Internal parasites and their effects on working equines in Morocco

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In a series of longitudinal studies in which 597 donkeys and 225 horses were examined, over 20 parasites were encountered : *Haemonema muscae*, *H. majus*, *Draschia megastoma*, *Trichostrongylus axei*, *Parascaris equorum*, *Strongyloides westeri*, *Strongylus vulgaris*, *S. edentatus*, *Oxyuris equi*, *Setaia equina*, *Gongylonema pulchrum*, *Cyathostome* species, *Anoplocephala magna*, *A. perfoliata*, *Paranoplocephala mamillana*, Hydatid cysts, *Fasciola hepatica*, *Dictyocaulus arnfieldi*, *Gasterophilus intestinalis*, *G. nasalis*, *G. haemorrhoidalis*.

Stomach worms (*T. axei*, *Haemonema* spp.), *Gasterophilus* larvae, *S. vulgaris* in arteries and gut, and *Cyathostomes*, were found in over 90% of animals examined, Polyparasitism involving several types of parasites in various organs was invariably observed. Although variations in epidemiological pattern occurred between different studies, as a whole, high levels of infections by most parasites were found in late autumn-winter, the cool rainy period of November-April. *S. vulgaris*, the most pathogenic parasite of equines, had an annual cycle with large number of larvae in arteries in spring and adults in gut in summer. The impact of parasitic infection was evident by poor body condition of working animals which was further aggravated due to intense agricultural activities during the period of high worm burden and poor nutrition. Benefits of 3 strategic treatments with dichlorvos in December, March (early and late rainy season) and July (early dry season) were evident by reduced faecal egg counts and upto 23% improvement in body condition. It is concluded that working equines in Morocco suffer from heavy polyparasitism and would benefit from control of these parasites.

Anthelmintic efficacy of doramectin against naturally occurring gastrointestinal nematodes of sheep

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The anthelmintic efficacy of four drugs viz., doramectin, ivermectin, fenbendazole and levamisole HCl was evaluated against naturally occurring gastrointestinal nematodes of sheep. All the drugs were administered at levels recommended by the manufacturers. Doramectin and ivermectin were found 100 percent effective against *Haemonchus* spp., *Trichostrongylus* spp., *Oesophagostomum* spp., *Bunostomum* spp., *Trichuris* spp. and *Strongyloides* spp. on the 7th day post-treatment. However, reappearance of *Haemonchus* spp. *Trichostrongylus* spp. and *Bunostomum* spp. eggs were observed from 35th day post-treatment onwards in sheep treated with ivermectin. Fenbendazole and levamisole were found 99.23 and 96.95 effective on day 7 post-treatment. Fenbendazole showed only 50 percent efficacy against *Bunostomum* spp., and *Trichuris* spp., 35.71 percent against *Oesophagostomum* spp., 25 percent against *Strongyloides* spp. and 1.87 percent against *Haemonchus* spp. Levamisole showed 80 percent efficacy against *Oesophagostomum*, 66.12 percent against *Haemonchus* spp. and no effect was observed on *Trichostrongylus* spp., *Bunostomum* spp., *Trichuris* spp., and *Strongyloides* spp. none of the drug showed any side effect.



Immunoreactive *Cryptosporidium parvum* antigens in cell culture media from infected MDBK cells

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Cryptosporidium parvum causes diarrhoea in ruminants and has been identified as the second most common infectious agent in calf diarrhoea. Immunoblotting with faecal IgA and serum IgG of convalescent *C. parvum* infected animals showed several soluble immunoreactive antigens in extracts of purified oocysts.

Monolayers of MDBK cells were grown in 24-well tissue culture plates at 1.5×10^5 cells per well in maintenance medium (8 % foetal calf serum [FCS], 2.2 mg NaHCO_3 , 200 U penicillin, 40 U gentamycin and 0.25 μg amphotericin B per ml MEM) at 37 °C in a 5 % CO_2 / 95 % air humidified incubator and inoculated after 24 h with 5×10^6 sporozoites in 400 μl of growth medium (4 % FCS). Full development of *C. parvum* was monitored by phase-contrast and electron microscopy. SDS-PAGE was performed on culture media concentrated on Centricon-10 (cut-off 10 kDa) after centrifugation at 200 g for 10 min. After Western blotting, the antigens were immunostained with specific anti-*C. parvum* faecal IgA and hyperimmune rabbit serum. The serum recognised a 31 kDa antigen in media from 24, 48 and 72 h infected cells, whereas faecal IgA recognised six antigens between 12 and 43 kDa. The culture medium also induced IFN- γ production by sensitised peripheral blood lymphocytes.

These data suggest that immunoreactive antigens may be produced in *C. parvum* cell culture medium.

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In vitro separation of pyrantel-susceptible and -resistant *Oesophagostomum dentatum* with a migration assay

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In order to separate pyrantel-susceptible and -resistant *Oesophagostomum dentatum*, a migration assay originally developed for the quantification of benzimidazole activity against *O. dentatum* was used. For the experiments adult worms were harvested from experimentally infected pigs and exposed to various concentrations of pyrantel for 30 minutes. Following exposure to the drug, the worms were transferred to migration chambers and allowed to migrate through polyamide net (mesh size 400 μm) for 30 minutes. The number of worms that had migrated through the net, and the number of worms retained on the net were counted. Using probit analysis, an EC_{50} of pyrantel for the sensitive isolate of 14.9 μm (95 % confidence limits : 11.5 to 19.4) was established. For the resistant isolate the EC_{50} was 253.8 μm (155.3 to 474.8). These values yielded a resistance ratio of 17.0. In a second series of experiments mixed samples of susceptible and resistant worms will be exposed to pyrantel and allowed to migrate. In a third series worms from pigs infected with both isolates will be used.

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The prevalence of helminths in different poultry production systems

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Two cross-sectional prevalence studies of helminths in poultry were carried out. The first cross-sectional prevalence study was conducted in populations of rural scavenging poultry on six hundred adult chickens selected randomly from six villages in the Morogoro Region, Tanzania during the wet and the dry seasons of 1995. The trachea and gastrointestinal tract of each bird were examined for the presence of parasites. All chickens were infested with one or several species of helminths the average being 4.8 (± 1.7) helminths per chicken during the wet season and 5.1 (± 1.8) during the dry season. A total of 29 different helminth species were shown in the trachea or the gastrointestinal tract. No trematodes were found. No correlation was found between season and prevalence, or season and mean worm burdens. Twelve helminths of the species recovered represent new local records.

The second study was conducted in Danish poultry production systems on 268 adult chickens selected randomly from 16 farms in Denmark from October 1994 to October 1995. The trachea and the gastrointestinal tract of each bird were examined for the presence of helminths and gave the following results : For the free-range/organic systems the following helminths were found : *Ascaridia galli* (63.8 %), *Heterakis gallinarum* (72.5 %), *Capillaria obsignata* (53.6 %), *Capillaria anatis* (31.9 %) and *Capillaria caudinflata* (1.5 %). For the deep-litter system: *A.galli* (41.9 %), *H.gallinarum* (19.4 %) and *C.obsignata* (51.6 %). For the battery cage system : *A.galli* (5 %) and *Railletina cesticillus* or *Choanotaenia infundibulum* (3.3 %). An exact identification of the cestodes was not possible due to missing scoleces. For the broiler/parent system: *C.obsignata* (1.6 %) and finally for the backyard system : *A.galli* (37.5 %), *H.gallinarum* (68.8 %), *C.obsignata* (50.0%), *C.anatis* (56.3 %) and *C.caudinflata* (6.3 %). The study confirmed the higher risk of getting helminth infections in free-range systems.

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The effect of insoluble dietary fibre on the establishment and persistence of *Ascaris suum* and *Oesophagostomum dentatum* in pigs

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This investigation compared the effect of four experimental diets formulated to provide increasing levels of insoluble dietary fibre on the establishment of *Ascaris suum* and *Oesophagostomum dentatum* in growing pigs.

Twenty-eight worm-free pigs, from a specific pathogen-free farm were randomly divided in four groups and were assigned to the following diets: diet A - barley flour plus protein (70%:30%); diet B - barley flour, oat husk meal plus protein (65%:7%:28%); diet C - barley flour, oat husk meal plus protein (60%:14%:26%) and diet D - barley flour, oat husk meal plus protein (55%:21%:24%). The diets were formulated to provide a constant levels of digestible protein per feeding units to pigs. All pigs were experimentally inoculated with 600 infective *A.suum* eggs and 6000 infective *O.dentatum* larvae and followed coprologically for 11 weeks post infection, whereafter they were slaughtered. Worm numbers, location, sex, development stage and worm fecundity were determined along with the concentration of insoluble (chromic oxid) marker, lignin and non-starch polysaccharides (NSP).

A.suum faecal egg counts and worm burdens were low and comparable in all diet groups. A significant difference ($P < 0.01$) in *O.dentatum* egg excretion was obtained between group D and groups A and B. The diet D was found to have a significantly higher worm burdens and fecundity of *O.dentatum* compared with the diets A and B ($P < 0.05$).

The present study suggests that a diet with increased content of lignin and insoluble NSP's (diet D) provides favourable conditions for the establishment of *O.dentatum* in the large intestine of pigs.



Anthelmintic efficacy of praziquantel 10% against the tapeworm *Anoplocephala perfoliata* in equids

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The activity of praziquantel 10% formulation was evaluated against *Anoplocephala perfoliata* in 5 naturally infected horses at a dose rate of 1.0 mg/kg. Faecal samples from a number of horses, suspected of harbouring tapeworms, were examined for the presence of tapeworm eggs using the centrifugation/flotation technique described by Proudman and Edwards (1992). 7 Horses were identified with tapeworm eggs present in the faeces, of which 5 were treated intraorally with praziquantel (0.01 ml/kg bodyweight) and 2 remained untreated as control animals.

The daily faecal output of each horse was collected for 4 days post treatment and macroscopically examined for excreted segments. Tapeworm segments were excreted by the five treated horses 2 to 4 days post treatment. No segments were recovered from the faeces of the untreated controls.

Four days post treatment the horses were necropsied. The small intestines and caeca of each horse were removed. The contents and mucosa scrapings were microscopically examined for *A. perfoliata*. The two untreated controls were found to be infected with *A. perfoliata* at necropsy. None of treated horses harboured *A. perfoliata* at post mortem. Some of the horses had lesions on the mucosa. Toxicosis was not evident in any of the horses post treatment.

Praziquantel 10% formulation at 1 mg/kg was 100% effective in eliminating *A. perfoliata* infestations from 5 treated horses.

Scanning electron microscopic study of *Anoplocephala perfoliata* in domestic equids

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The genus *Anoplocephala*, the origin of the mammalian anoplocephalids, occurs in a wide range of mammals, possibly suggesting a long association between hosts and parasites. *Anoplocephala* appears to have a number of ancestral characteristics, but which, if any, of the current hosts of the genus were the original ones is not clear.

Anoplocephala perfoliata is the most common cestode species occurring in domestic equids. A number of specimens was recovered at necropsy from the small intestine and caecum of a horse and prepared for scanning electron microscopy. The photographs reveal the external and internal morphology of mature tapeworms, as well as the posterior proglottids of an immature tapeworm.

The immature tapeworm is wide anteriorly and tapers posteriorly. The first-formed proglottid with a terminal bladder is still attached to the developing proglottids. The posterior proglottid has a central opening for the excretory tubes.

Mature *A. perfoliata* has a globular scolex with a flattened anterior surface. The lack of a rostellum and any form of armature on the four spherical suckers, is characteristic of the family Anoplocephalidae. The posterior edge of the scolex has a pair of lappets on each side.

Well-defined proglottids begin closely behind the scolex. Proglottids are wider than long, markedly craspedote. The external covering is composed of a perforated cuticle with small pores. The muscles and organs are imbedded in a parenchyma. The cortex is provided with two layers of longitudinal muscles. A gravid proglottid shows the uterus packed with eggs, filling the proglottid.

Strategic control programme of avian malaria in broilers

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Outbreaks of avian malaria due to *Plasmodium gallinaceum* in broilers have been evident in Thailand since late 1995. The control measures of the disease have been designed to reduced the morbidity and mortality rates of naturally acquired infected animals. Throughout the experiment, eleven thousand 3-day-old chicks were raised and allowed to expose to the malarial parasites in a private farm under local environmental conditions of known endemic area for avian malaria. Chloroquine phosphate was a drug of choice given to the whole flock in water at a dosage of 5mg/kg body weight/day for 3 consecutive days. Thereafter, the drug was repeatedly given 10mg/kg body weight/day in the same manner to the chickens at the ages of 10, 20, 30, 40, 50 and 60 days old. Parasitaemia, haematocrit value, clinical signs and % death of 150-250 chickens were randomly observed on 3, 9, 16, 26, 36, 46, 56 and 62 days old. Our investigation showed that malarial parasites were not found in 3 to 26 days old animals and a low parasitaemia was demonstrated between 0.5-3.8 % on days 36 onward. Changes of haematocrit value was not clearly seen but morbidity and mortality rate of treated animals was significantly reduced. Avian malaria control programme using chloroquine phosphate administration is likely a great advantage to prevent economic loss in poultry industry.

The effect of diet composition on the anthelmintic activity of piperazine dihydrochloride against nodular worms in pigs.

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An in vivo study was carried out in attempt to evaluate the effect of diet composition on the efficacy of piperazine against nodular worms of pigs. Twenty pigs were each administered 3,000 infective larvae of mixed isolate of *O. dentatum* and *O. quadrispinulatum*. Twenty-one days later the pigs were divided into 5 groups according to their epg counts. Beginning day 23 post-infection, pigs in groups 1 and 2 were fed a low fibre diet consisting of 70 % barley flour and 30 % protein concentrate while those in groups 3, 4 and 5 were fed a high fibre diet, supposed to have a faster gastrointestinal transit time, consisting of 55 % barley flour, 21 % oat husk meal and 24 % protein concentrate. Pigs in all groups were reinfected with the same number of *Oesophagostomum* spp. larvae 37 days after their first infection. On day 42 following the initial infection, pigs in selected groups were administered oral piperazine dihydrochloride 53 %: groups 1 and 3 received 200 mg/kg bodyweight while pigs in group 4 were given 100 mg/kg. Groups 2 and 5 served as non-treated controls for the respective diet regimens. Eight days after treatment (50 days after the first infection), the pigs were slaughtered and worms recovered from the large intestine and counted. The mean worm count reduction (WCR) in group 1 (full dose piperazine with low fibre diet) was 89.8% while high fibre diet in group 3 increased the WCR to 99.4%. In group 4, where the pigs were fed the high fibre diet and treated with only 50 % dose of piperazine, the WCR was 90.9% which was not statistically different from "low fibre" group 1 treated with full-dosed piperazine. That indicates that increased digesta flow rate carried the drug faster through absorptive sites of small intestine thus reducing the quantitative absorption which resulted in a greater amount of piperazine reaching the large intestine, the site of *Oesophagostomum* infection. This work has conclusively demonstrated that modifying the physiological-pharmacological relationship using modified diets provides effective parasite control using piperazine. This procedure can be one of the options how to maintain or improve chemotherapy in future.



Efficacy of doramectin pour-on against the eyeworm *Thelazia rhodesi* in naturally infected cattle

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Thelazia spp., a widespread group of spirurid parasites found in the eyes of mammals, has been shown to cause a variety of clinical signs including photophobia, conjunctivitis and keratitis.

A study was conducted to evaluate the efficacy of doramectin pour-on against *T. rhodesi*. Nineteen one- to 11- year-old podolic cattle naturally infected with *T. rhodesi* and grazing on a farm in south-eastern Italy (Gargano peninsula, Apulia region) were used. Cattle were randomly allocated to either a doramectin-treatment group (nine animals) or a non-medicated control group (ten animals). The treatment group received doramectin pour-on at a dose of 500 µg/kg (1 mL/10 kg of body weight) while the control group received no treatment. Animals were penned so that no physical contact was possible between the two groups. No adverse reactions or clinical signs were observed in cattle at any time during the study. All animals were slaughtered 14 to 15 days after treatment, and the eyes and associated tissues (including lacrimal glands and ducts) were removed and examined for total number, species and viability of eyeworms.

Non-medicated animals with no worms and an equal number of treated animals were excluded from summary and analyses. A log transformation ($\ln(x+1)$) was applied to the worm counts prior to analyses. Efficacy was calculated based on percentage reduction in worm burdens of treated cattle compared to non-medicated animals. No worms were recorded in the doramectin-treated animals; live adults of *Thelazia rhodesi* were found in nine out of ten untreated control cattle, with parasite burdens ranging from one to 14 worms. The efficacy of doramectin against *T. rhodesi* in naturally infected cattle was 100% ($P=0.0001$) following a single application of doramectin pour-on at a dosage of 500 µg/kg.

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Effect of sex and parasitic diseases on the camel meat composition

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Meat samples of normal and parasitic infested camels were obtained from the slaughter house and analysed for biochemicals, trace and toxic elements. Camel meat infested with *Cysticercus dromedarii*, hydatid cysts, and sarcocystis was identified. Parasitic infested meat revealed a significant increase in the water content and decrease in the protein, fat and ash contents as compared to normal meat. Dressing percentage obtained from male camel meat (73%) was higher than female (64%). Water content was 76.29, protein 21.03, fat 0.94, and ash 1.19 percent in normal meat. The proportion of chemical composition of camel meat was not influenced by sex. However, water contents were slightly more in female as compare to male. Arsenic, cadmium, selenium and iron concentrations were higher in kidney as compared to liver and muscles, while mercury, copper, zinc and manganese were higher in the liver. Male had a significantly higher carcass weight than female. Present study indicate that parasitic infestation does effect on the meat quality characteristics.

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Isolation and *in vitro* cultivation of *Histomonas meleagridis*

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199 medium-plus chicken embryo extract and horse serum was used to isolate *Histomonas meleagridis* from cecal contents of 23 turkeys with natural histomoniasis. After 12-24 hours of incubation at 39°C, active *H. meleagridis* could be observed under phase microscope, and the isolation rate was 65.8%. *Trichomonas* spp. were also present in the culture tubes from some cases (34.8%). Gentamycin (400-800u/ml) facilitated the initial isolation of *H. meleagridis*. Some factors affecting the growth of *H. meleagridis in vitro* were tested in the successive cultures with same medium. The initial pH of medium showed significant effects on the growth of the protozoa. The number of protozoa in the medium with initial pH 8.0-8.5 showed significantly ($P < 0.05$) higher than that in the medium with initial pH 6.5, 7.0 and 9.0. When the initial pH was lower than 6.5 or higher than 9.0, the parasite could hardly grow in the medium. *H. meleagridis* showed significantly ($P < 0.05$) better growth and live longer in anaerobic condition than aerobic state. Adding rice starch to the medium was proved to be necessary for the growth of *H. meleagridis in vitro*, and the starch grains could be observed within some of the parasites.

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Pathophysiology of *Echinococcus granulosus* infected camel.

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A total of 390 camels irrespective of their age and sex were examined for pravelance, fertility ratio, immunodiagnosis, biochemical and enzymatic profiles of hydatid cystic fluid of camels. Indirect heam-agglutination test (IHA) have shown to be sensitive in 90.32% in serum of infected camel. IHA test was more sensitive in detecting hepatic cyst (85%) as compare to lung cyst (83.33%). The incidence of disease in camel was recorded to be 65.50 percent. Lungs were found to be the most common infected organ in both sex, and its pravealance was recorded to be 100 percent in the lungs. The number of cyst were 2-20 in lungs, 1-30 in liver and 1-5 in spleen. The size of cysts in lungs ranged from 2-35 cm, 1-40 cm in liver and 1-20 cm in spleen. The cystic fluid was randomly collected from liver, lungs and spleen. The pH value of hydatid cystic fluid of liver and lungs differ from each other. Glucose, proteins and lipids content of cyctic fluid were significantly higher in liver as compared to lung. Chloride, iron and copper concentration of liver cystic fluid were higher. The sodium were found to higher as compared to potassium in liver than lung cystic fluid.

Ectoparasites of Camel: Haematochemical and therapeutic studies on Sarcoptic Mange.

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Camels were examined to determine the prevalence and to identify the species of ectoparasites on camel. 194 (37%) were found to be infested with external parasites. Only one species of mite *Sarcoptes scabii* var camels was recorded in adult camel. Higher incidence (68%) of mange was recorded in adult camel. Incidence of mange was found to be more in male (56%) than female (49%). During hot humid season the mange infestation was highest (57%) followed by winter (53%). Infested camel showed slight decrease in erythrocyte, Hb and PCV. A significant increase in white blood cells was followed by an increase in neutrophils, eosinophils and decrease in lymphocytes. Serum biochemical study revealed hypoalbuminaemia, hyperglobulinaemia. Increase in GOT and ALP was evident, while GPT decreased significantly. The assessment of efficiency of the Ivermectin (0.4 mg/kg bw) was on the basis of parasitological recovery.

Persistent efficacy of eprinomectin pour-on against gastro-intestinal and pulmonary nematodes in cattle

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A topically applied formulation of eprinomectin, a novel, highly effective macrocyclic lactone, has been developed as an endectocide for beef and dairy cattle. Six studies were conducted to assess persistent efficacy of the product by challenging groups of animals with infective third stage nematode larvae at a specific time after treatment and by comparing parasite burdens at necropsy. Investigations were conducted in Australia, Germany, the United Kingdom and the United States and included 159 young cattle of both sexes, various breeds and weighing 86 to 254 kg. Groups of at least 6 animals (4 in one study) were treated topically along the backline at the recommended dose level of 500 mcg/kg or left as untreated controls. Challenge with infective larvae was made at 7, 14, 21, 28 or 35 days after treatment. Overall, persistent activity was evaluated against 12 species of gastrointestinal nematodes and lungworm, representing 7 genera. All data were combined, and weighing each trial equally, the persistence period was established based on a significant ($p < 0.01$) reduction of $> 90\%$ in geometric mean counts of the treated groups compared to controls. Results demonstrate that eprinomectin applied topically at 500 mcg/kg controls infections of *Haemonchus placei* and *Trichostrongylus* spp (including *T. axei* and *T. colubriformis*) for 21 days after treatment and *Ostertagia* spp (including *O. ostertagi*, *O. lyrata* and *O. leptospicularis*), *Cooperia* spp. (including *C. concophora*, *C. punctata* and *C. surnabada*), *Nematodirus helvetianus*, *Oesophagostomum radiatum* and *Dictyocaulus viviparus* for 28 days after treatment. Reductions greater than 90% were also obtained 35 days after treatment for *Nematodirus helvetianus* (94.7%) and *Dictyocaulus viviparus* (96.9%), although they were tested in one study only for this timepoint.

Evaluation of the efficacy against L₄ stages and the persistent efficacy of moxidectin 0.5% pour-on against *Ostertagia* spp. and *Dictyocaulus viviparus* in cattle.

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This study was conducted to evaluate the efficacy against L₄ stages and the persistent efficacy of moxidectin 0.5% pour-on against experimental challenge with infective larvae of *Ostertagia* spp. and *Dictyocaulus viviparus* in cattle. A group of 48 calves, negative for trichostrongyle-type eggs and for lungworm larvae on fecal examination, were randomly assigned to 6 groups of 8 calves each based on body weight. Four groups of calves were each treated with moxidectin at a dose rate of 500 µg/kg (0.1 ml moxidectin pour-on formulation/g body weight) at weekly intervals on Days -42, -35, -28 and -21 prior to infection. The fifth group remained as untreated controls. The final group was treated on Day 5 postinfection at the same dose rate. On Day 0 each calf was infected with 25,000 *Ostertagia* spp. and 1,500 *D. viviparus* infective larvae. All calves were necropsied 28-30 days postinfection. The abomasum and lungs were removed from each animal and processed for nematode recovery. Geometric mean nematode counts of *Ostertagia* spp. and *D. viviparus* were calculated from log (counts + 1) and used to estimate percent reduction. In this study, moxidectin 0.5% pour-on was 100% efficacious against L₄ stages of both *Ostertagia* spp. and *D. viviparus*; the persistent efficacy against *D. viviparus* was 100% for at least 42 days posttreatment. The persistent efficacy against *Ostertagia* spp. was $>99.9\%$, 99.9%, 95% and 92% when challenged 21, 28, 35 and 42 days posttreatment, respectively.

Survival of infecting (L3) larvae of *Cyathostominae* under extreme temperature and humidity conditions.

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The duration of pasture contamination with cyathostome larvae varies in different climates, but is generally long. Light, high temperatures and lack of humidity are presumed detrimental for L3 survival. In order to obtain data that may predict the length of contamination in any climate and suggest methods for cleaning pastures, L3's were submitted to extreme physical conditions and their survival was studied. Larvae were obtained from: a) Coprocultures b) Naturally infected, dry reservoirs: manure and grass, collected from a pasture free of horses during 2 rainless months and c) laboratory infected faeces and hay with no environmental contamination. All samples were kept indoors, with direct natural lighting and no air conditioning, for the duration of the experiments. Survival was determined by wetting the sample with H₂O and observing L3's under microscope: only larvae with active movements were classified as alive.

Living L3's were consistently recovered from dry natural or laboratory infected faecal and grass samples for the duration of the experiments (19 months to date). Samples of faeces were wetted and either submitted to weekly dry-wet cycles at room temperature or kept soaked until all larvae were dead. The amount of surviving larvae decreased to 50% only after 20 cycles, and about 5% survived at least 30 cycles. In contrast, L3 survived less than 30 days when continually soaked. Coproculture larvae were suspended in H₂O or PBS and subjected to dry/wet cycles at different temperatures; after each cycle the percentage of living larvae was calculated. In all cases, there were motile larvae after 3 or more cycles; the best survival rates were obtained with larvae suspended in saline and at 30 °C. Finally, coproculture larvae were seeded over sterile faeces and submitted to dry heating for 1 h. Perfectly motile L3's were recovered after being submitted to temperatures up to 55 °C, but not at 60 °C.

Our results show that, a) although a proportion of larvae may die when dried, many dry L3's survive for at least 1.5 years; b) larvae may resist much longer (30 weeks) in wet/dry situations in their natural reservoir than when constantly drenched; c) L3's survived better when dried in a saline medium; and d) very high, dry temperatures do not kill them. They explain the pastures' contamination persistence of in all kind of weather and suggest that only flooding a pasture during at least 20 days in hot weather may clean it.



Feline Heartworm Infections, A Clinical Survey in the USA

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Diagnosis of feline heartworm disease can be elusive due to non-specific clinical signs, typically low worm burdens, unique pathophysiology in the cat and the limitations of currently available heartworm tests. Consequently, knowledge about this disease is still limited. The objective of this study was to survey the occurrence and clinical presentation of feline heartworm infection in specific regions of the United States among cats presenting with signs consistent with heartworm infection. Submission of 200 cases from fifteen private practices in Florida, South Carolina, Tennessee and Texas was targeted. Cats entered in the study were at least 6 months of age and presented with one or more of the following clinical signs: respiratory signs, including tachypnea, dyspnea or coughing; gastrointestinal signs, including a pattern of intermittent vomiting unrelated to eating; or sudden death of uncertain etiology, particularly associated with respiratory distress prior to death. Data collected included: history and indoor/outdoor lifestyle; physical examination findings; thoracic radiography evaluations; Knott or DIFIL[®] test results, DIROCHEK[®] antigen test results, antibody test results (Animal Diagnostics, Inc. and Heska Corporation laboratories); and CBC results. Preliminary analysis of 162 cases provided the following results: 68/162 (42%) tested antibody positive on one or both antibody tests; 16/68 (24%) antibody positive cats were reported to be indoors 100% of the time; and 21/68 (31%) antibody positive cats spent $\leq 10\%$ of their time outdoors. Six of the 162 were antigen positive; one other cat from which blood was not obtained was diagnosed positive at necropsy. In 24 of the cases, thoracic radiographs showed signs consistent with heartworm infection.

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Whirling disease (*Myxobolus cerebralis*) of salmonid fishes in the USA: current impacts and research

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Whirling disease of salmonid fish is caused by the parasite *Myxobolus cerebralis*. The sporogonic stage of the parasite destroys cartilage, producing a characteristic whirling behavior, among other symptoms. This parasite was first detected in Pennsylvania, USA in 1958, probably introduced from frozen European trout. It has been found in at least 14 states since. Whirling disease has been found in enough trout populations in either hatchery-reared trout or native trout populations to be considered a serious economic problem. In Montana, the disease was first reported in 1994 after an unusually high decline in rainbow trout (*Oncorhynchus mykiss*) was reported for one of its principal "blue-ribbon" trout streams. Because Montana maintains wild trout fisheries, the presence of this disease has great significance.

As other states have begun reporting whirling disease epizootics, the research effort has increased markedly with support coming from a variety of sources. Current areas of interest include: improved procedures for disease diagnosis or detection of the various parasite life cycle stages, fish genetics for disease-resistant species, parasite distribution studies, parasite life cycle studies including seasonal transmission, and others.

The study undertaken by our laboratory involves an analysis of alternate host populations at an enzootic site in Montana. Samples of aquatic oligochaetes consist primarily of *Tubifex tubifex*. This oligochaete is regarded as the principal, if not the sole, alternate host for the whirling disease organism. A PCR-based test, developed at the University of California-Davis, has been used to detect *M. cerebralis* rDNA in oligochaetes. Using this test we have been able to detect the presence of the parasite in oligochaetes from the study site. Preliminary results indicate an infection prevalence of ca. 1%. A similar prevalence has been found in observations of oligochaetes shedding the triactinomyxon stage of the parasite. In year-long collections at biweekly intervals, abundant numbers of *T. tubifex* have been found although a seasonality of transmission has not been determined.

Ascaris suum population kinetics in pigs following single inoculations with different numbers of infective eggs

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To study population kinetics during primary *Ascaris suum* infections, three groups of 52 pigs each were inoculated with 100, 1000, or 10000 infective eggs. The worm numbers on 10 different days post inoculation (p.i.) were obtained using an agar-gel technique on the liver tissue and the small intestinal contents, and a macrobaermann method on the lung tissue. In all groups, the majority of larvae were found in the liver on day 3 p.i. and in the lungs on day 7 p.i. Liver white spots, caused by migrating larvae, were most numerous at day 7 p.i., whereafter they gradually healed, and only low numbers of granulation-tissue type white spots and lymphonodular white spots persisted at days 21-56 p.i. Independent of dose level, 47-58% of the inoculated eggs were recovered as larvae in the small intestine on day 10 p.i., but most larvae were eliminated at days 17-21 p.i. This elimination started earlier and removed a higher percentage of the worms with increasing inoculation dose, resulting in small strongly aggregated worm populations by day 28 p.i. (*k* of the negative binomial distribution was low: 0.2-0.4) without significant differences between groups. Thus, overdispersion, which is a characteristic of both porcine and human ascariasis, is found here under experimental conditions where aggregation factors like host behaviour, transmission rate, host status etc. have been partly or totally controlled.

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REVIEW ON THE PROGRESS IN BOOPHILUS MICROPLUS (CAN.) ERADICATION PROGRAM IN ARGENTINA.

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The cattle tick *Boophilus microplus* (Can.) is the most important ectoparasite affecting livestock in the sub-tropical areas of Argentina.

Since its introduction in Argentina on last century *Boophilus microplus* spread up to 34th parallel of latitude Sur and in the first year of this century it was estimated that about one million square kilometers were infested.

For its control severe measures were taken to prevent spread to southwards regions and on 1938 a compulsory eradication program was established by Government.

The Country was divided into four areas: Infested area; Preparatory Control area, Control area, and Tick free area. According to this strategy the farmers had been obliged to periodic compulsory plunging dips with Ixodides and to a quarantine before removing cattle from one area to another. Different acaricides had been used as arsenic compounds, BHC derivatives, organophosphates and on last years the most employed tickicides were synthetic pyrethroids but recently resistance has been detected at different levels and now farmers are using Amitraz.

The results obtained applying this National Program for Tick Eradication were very successful and up to day *Boophilus microplus* had been eradicated over 290.000 square kilometers and meat production increased 300.000 tons.



TICKS INFESTING DOGS IN BUENOS AIRES METROPOLITAN AREA.

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Throughout 1996 and in the first months of 1997, were examined 578 dogs in order to determine genus of ticks that were infesting dogs in Buenos Aires metropolitan area.

This study was carried out on dogs living in flats and houses, as well as in slums, suburban and field areas, kennels and asylums for street dogs.

It is known that *Rhipicephalus sanguineus*, the brown dog tick, is the most wide spread tick all over Argentina from subtropical regions to Patagonia, and was introduced to the Country from Brazil.

The first specimen were observed and recovered in 1941 in Buenos Aires metropolitan area and in the rural regions near Brazilian borders.

In this study adult and immature stages of *Rhipicephalus sanguineus* were found on 100% dogs examined and according to these findings it was estimated that *R.sanguineus* is completing 3 life cycles yearly in a temperate zone such as the Buenos Aires metropolitan area.

In infested kennels as well as in houses situated in slums or in field areas, it was possible to observe newly active adults and nymphs climbing walls from floor-level cracks and remaining stationary on walls near the roof.

In this last years due to migration of people from rural regions to Buenos Aires metropolitan area genus *Amblyomma spp.* was introduced.

Conclusions: In the Buenos Aires metropolitan area *Rhipicephalus sanguineus* is the most common tick but in this study also *Amblyomma fozsum* and *Amblyomma tigrinum* were observed.

IMIDACLOPRID, A NEW INSECTICIDAL COMPOUND, AGAINST CTENOCEPHALIDES SPP. ON NATURALLY INFESTED DOGS.

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From January to March 1996 two field studies were carried out in a naturally fleas infested asylum for dogs, situated in a western suburb of Buenos Aires metropolitan area, in order to determine Imidacloprid efficacy, tolerability and post-treatment protection period against naturally fleas reinfestation. A 10% w/v spot-on formulation was investigated against *Ctenocephalides spp* on 72 naturally infested dogs living in private canine asylum.

Dogs were randomly allocated into two group: Group 1 (65 dogs) was treated and Group 2 (7 dogs) acted as untreated control. Imidacloprid was applied, as a single spot-on, at the dosage rate of 1 ml/10 kg b.w. (10 mg/kg.b.w.), on the skin between the shoulder blades on dogs weighing from 4 to 10 kg.; on large dogs, weighing more than 25 kg., the dose volume was split and only half was applied between the shoulder blades and the other half on top of the rump. Post treatment flea counts were carried out at 24 hs and on day 7, 14, 21, and 28 days after applications and the flea counts between the treated dogs and the untreated animals were compared. Based on flea counts after the first application the efficacy at 24 hours after the treatment was 100%; on day 7 was 99.04%, on day 14 was still 100%; on day 21 was a 98.9%, and on day 28 was a 98.3%. A second Imidacloprid spot-on application was carried out on day 35 and the product efficacy at 24 hours, on 7 days and on day 14 was 100%; on day 21 was 99.4 and on day 28 was 98.9%.

Conclusions: According to these results Imidacloprid spot-on 10%, at 10 mg/kg b.w., demonstrated that can cut off the development cycle of a flea population highly infesting an environment without using additional measures, and provided a very effective protection against *Ctenocephalides spp.* reinfestation.

IMIDACLOPRID SPOT-ON 10% FOR THE CONTROL OF CTENOCEPHALIDES FELIS ON NATURALLY INFESTED CATS.

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From November 1996 to February 1997 the efficiency of Imidacloprid spot-on 10% was investigated against *Ctenocephalides felis* on 36 naturally infested cats lodged in a private asylum for cats situated in Buenos Aires suburban area. Imidacloprid was applied at a single dose of 10 mg/kg in one spot directly onto the skin on mid-line of the upper part of dorsal neck. The applied dose volume was 0.4 ml for cats $\leq$ 4 kg b.w. and 0.8 ml for cats $>$ 4 kg.b.w.

Cats were randomly allocated into two groups, one treatment group (30 cats) and an untreated control group (6 cats). Post treatment flea counts were carried out at 24 hs. and on days 7, 14, 21, 28, and 35.

Product efficacy was calculated by comparison flea counts on treated group with that on control group. The results obtained in the check-ups demonstrated a 100% efficiency 24 hs. after application; in the following check-ups the efficiency was 99.2% on day 7; 98.1% on day 14; 97.3% on day 21; 95.9% on day 28 and 89.7% on day 35.

Imidacloprid spot-on application had been repeated on day 41 and also after this second treatment flea counts were carried out at the same day intervals as after the first application.

According to these results also a 100% efficiency was obtained 24 hs. after this treatment, and in the following check-ups it was 99.7% on day 7; 98.3% on day 14; 97.9% on day 21; 96.7% on day 28 and 95.1% on day 35.

Conclusions: Based on these results Imidacloprid spot-on 10% at 10 mg/kg b.w. controlled very well *Ctenocephalides felis* on cats and also cut off the development cycle of a flea population infesting the environment in a cat asylum during the warmer months in South American summer.

ECONOMIC LOSSES DUE TO BOOPHILUS MICROPLUS (CAN.) IN ARGENTINA. PERIOD 1994 -1996.

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Cattle production is one of the most important activities in Argentina and among the ectoparasites affecting livestock the cattle tick *Boophilus microplus (Can.)* is the most important in the subtropical region where about 12.5 million cattle are grazing.

During the period 1994 - 1996 losses due to *Boophilus microplus* had been estimated yearly in US\$ 112 million. Physic losses due to tick infestation (as loss of live weight and the corresponding percentage of expected meat gain, reduction in milk production, mortality and calving reductions, hide damages) accounted for US\$ 70.56 million (63%).

Losses due to mortality and morbidity for bovine haemoparasites (*Babesia* & *Anaplasma*) transmitted by *Boophilus microplus* had been estimated in US\$ 21.28 million (19%).

The expenses for *Boophilus microplus* control (as tickicides, manpower, plunging dips, National tick eradication program,) accounted US\$ 16.8 million (15%) while the expenses for *Babesia* & *Anaplasma* control had been estimated in US\$ 3.36 million (3%).

LOSSES DUE TO TROPICAL WARBLE-FLY (*DERMATOBIA HOMINIS*) IN NORTHERN REGIONS OF ARGENTINA.

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Dermatobia hominis is widely distributed from Mexico to the northern regions of Argentina and is one of the most important parasites of cattle in Latin America causing annual losses estimated in US\$ 260 million.

In Argentina the endemic area is 58,627 Km², corresponding to the province of Mission and to the grazing lands bordering the Paraná, Uruguay and Paraguay rivers in the nearby provinces.

In this area about 1,200,000 cattle are grazing and during the fly season from September to April, the tropical warble-fly is causing severe economic losses to the farmers reducing meat and milk production.

It was observed in a heavily infested herd that a steer with 20 to 40 grubs loses from 10 to 12% of its weight and in cows milk production decreases up to 20%.

Meat packers estimated that the lesions caused by parasites and secondary bacterial contamination require about a 5 kilos meat trimming.

The 65% of the checked hides in Mission and in the nearby areas of Corrientes province had holes due to *D. hominis* larvae perforations and are useless for sale to tanneries or for manufacturing of rural implements.

Besides domestic and wild animals *D. hominis* may affect man especially children and working people in rural area.

Up to some years ago prevention of this myiasis involved use of chlorinated hydrocarbon insecticides and synthetic pyrethroids in sprays, dips and pour-on; now the best control is obtained injecting cattle with avermectins (Ivermectin and Doramectin).

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Genetic resistance to the sheep parasite in two Dutch sheep breeds : the Flevolander sheep and the Texel sheep

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Haemonchus contortus is a harmful nematode parasite of sheep. Increasing innate resistance in sheep to this parasite will reduce the need for anthelmintic treatments. As about 50 % of the parasites on the field is caused by about 10 % 'wormy sheep' it is important to be able to identify these sheep to exclude them from breeding. The development of molecular markers for non-invasive identification of parasite-resistant or parasite susceptible sheep is prerequisite for this type of selective breeding. Recently some molecular markers were identified but more markers in other parts of the genome are needed as parasite resistance is a trait caused by several factors. We started to analyse two sheep breeds for resistance to *H. contortus* that had never been investigated for this trait before. These breeds were maintained for 25 years in our Institute and the pedigree lines monitored. 50 sheep of each breed, age 6-12 months were infected with 2500 L3 larvae. The eggs per gram (epg), haematocrit and weight were monitored once a week. After 7 weeks a second infection followed with 5000 L3 larvae. The epg's in the Flevolander sheep were 3-5 times as high compared to the Texel sheep. Differences in effects on the haematocrit and weights were also found. The two breeds will be the starting material for further research on genetic markers for resistance in sheep to *H. contortus*.

Anthelmintic efficacy of ivermectin after its subcutaneous administration to calves naturally infected at dose rates of 4 and 8mg/kg

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Ivermectin (ABZ), is widely used as an anthelmintic in ruminants. ABZ activity is mainly due to its metabolism to sulfoxide (SO) and sulfone (SO₂). ABZSO is known as ivermectin (RBZ), and is the drug that reach the highest plasma concentrations after ABZ administration to cattle. Injectable RBZ formulations for subcutaneous administration were recently introduced in the Argentine market. The objective of the present paper was to characterize the anthelmintic efficacy of RBZ against gastrointestinal parasites with special emphasis in *Ostertagia ostertagi* L4 inhibited larvae naturally acquired, after its SC administration to cattle at doses of 4 and 8mg/kg.

Eighteen 15 to 18 months old castrated male Aberdeen Angus bovines weighting 150-250kg naturally infected with trichostrongyles in the Humed Pampa of the Buenos Aires Province were used in the present experiment. After 16 days of permanence in concrete floor corrals and feed lucerne hay, the experimental animals were treated according to the following scheme : 6 injected subcutaneously with 4mg/kg ivermectin 10 % (Sintyotal R (r), Biogénesis-Sintyal, Argentina), 6 injected by the same route with the same pharmaceutical at a dose of 8mg/kg, the remaining six being kept as controls. Seven days after the administration, the experimental animals were sacrificed parasite counts being performed in abomasum, small and large intestine looking for adults, and after peptic digestion of abomasum mucosa looking for L4 *Ostertagia* larvae.

Results were analyzed by comparison of geometric means for each parasite species in each group, the percentage of control for the different species ranging between 85.61 % for L4 *Ostertagia* larvae and 100 % for more sensitive parasites in the case of the 4mg/kg dose and between 94.55 % for L4 *Ostertagia* larvae and 100 % for more sensitive parasites in the case of the 8mg/kg dose.

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The epidemiology of gastro-intestinal nematodes in dairy cattle in the Lake Victoria basin (Uganda).

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To establish an effective control strategy for gastro-intestinal nematodes in dairy cattle in the Lake Victoria basin, analytical observational studies were utilized to establish associations between helminthiasis and the parameters of age, season and grazing systems. Also ecological studies of the developmental stages on pastures were carried out.

There was a chronological development of infection *Strongyloides papillosus* being the earliest. Age and grazing systems influenced prevalence, intensity and spectrum of infection. The most susceptible age bracket was 4 to 12 months old. The distribution, survival and availability of infective stages (L₃) was determined by the amount and frequency of rainfall. There was poor translation during the dry spells.

The Exclusive and Criss-Cross grazing systems should be avoided while Rotational and Calf-Dam systems should be encouraged. For cost-effectiveness, critical and strategic anthelmintic treatments should be concentrated on the 4-12 month age bracket.



Pattern of infection with gastrointestinal nematodes in a highland area of Kenya

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An epidemiological study to establish the pattern of infection of gastrointestinal nematodes of ruminants was conducted in 27 small scale farms with a total of 150 cattle, 120 goats and 50 sheep in the Kericho highlands of western Kenya for a period of 22 months. Monthly faecal egg counts were performed on all animals. Tracer sheep were introduced to monitor larval contamination while local sheep were purchased and slaughtered to determine the worm loads at different times of the year. Pasture samples along the grazing site were taken monthly for pasture larval recovery and identification and pooled faecal samples from each host species were cultured to identify the species of nematode present in the individual farm. The pattern of infection in all the species varied with cattle having average low faecal egg counts (95-638) whereas the sheep and goats showed high counts with ranges of 372-3638 and 268-1899 respectively. The peaks in all the species were in April to May and November to December. The pasture larval counts showed presence of gastrointestinal parasites' larvae with *Haemonchus contortus* being predominant throughout the year with peaks in April to June.

Both the tracer lambs and the locally purchased ewes showed infections throughout the year with *H. contortus* and *Trichostrongylus* species being the predominant respectively. The differential larval counts in all ruminant species showed the commonest species were *Haemonchus* and *Trichostrongylus* with traces of *Oesophagostomum*, *Cooperia* and *Nematodirus*.

The results of this study will enable appropriate intervention to be instituted in the study area for control of gastrointestinal parasites.

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Lack of protective activity of soluble parasite antigen from *Babesia canis rossi* parasites

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Dogs can be vaccinated against clinical babesiosis due to *B.c. canis* using vaccine preparations containing soluble parasite antigens (SPA) derived from in vitro culture. Vaccine efficacy appeared to be associated with the effect on SPA levels in plasma after challenge infection, and not necessarily with an effect on the peripheral parasitaemia. Using two different *B. canis* strains from Europe the peripheral parasitaemia after primary infection was low (less than 1%), in contrast when using a South-African isolate, parasitaemia developed exponentially to levels much higher than 1%. The etiology of disease following infection with European *B.c. canis* and South-African *B.c. rossi* appeared to be different. In *B.c. canis* infection disease was correlated with increased coagulation time (APTT), whereas in *B.c. rossi* infection disease was correlated with the peripheral parasitaemia. When dogs were vaccinated with SPA from either *B.c. canis* or *B.c. rossi* parasites, it appeared that vaccination against clinical disease was apparent in the former, but not in the latter group. The results suggest that the vaccination principle using SPA is effective in infections characterised by low parasitaemia and an effect on the coagulation system, and not in infections characterised by exponentially developing parasitaemia. This also suggests that SPA produced by *B.c. canis* parasites cause disease, possibly by activating the coagulation system, and that vaccination against SPA primes animals for the production of SPA-blocking antibodies.

Nematode development: Control by amphidial neurons

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The parasitic nematode, *Strongyloides stercoralis*, and the free living nematode, *Caenorhabditis elegans*, can develop by similar, alternative, life history pathways. *S. stercoralis* can follow a direct pathway having only 3 free living larval stages, the third being a non-feeding, developmentally arrested, infective stage (L3), i.e., a dauer larva homologue. Alternatively, it can follow an indirect cycle, with 4 larval stages the last giving rise to either a free living adult male or female. These give rise to a succession of larval stages (L1- L3), the last being infective. Although there is a heritable propensity to develop mainly by one of these routes, environmental factors affecting the parasitic female or its early-stage progeny can also determine the route followed. In *C. elegans*, crowding and the amount of food available to the L1 are the environmental factors that determine whether a dauer larva (a developmentally arrested L3) is included in the life cycle. These environmental factors are sensed by two amphidial neurons, which, in turn, control the route of development. A third controls resumption of development by individuals in dauer arrest. We have traced each of 13 amphidial neurons of *S. stercoralis* to its cell body and determined possible homologies with like neurons in *C. elegans*. The positions of these bodies and their neighbors were recorded to make a map for laser killing of individually recognized neurons. Then, by neuronal ablation we have sought to determine whether functionally similar amphidial neurons occur in *S. stercoralis* and whether other chemosensory amphidial neurons are involved in host-finding behavior.

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Differences in gene expression of arrested and non-arrested larvae of *Dictyocaulus viviparus*

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Arrested development (hypobiosis) is one of the most important strategies for nematodes to survive periods of unfavourable environmental conditions. To investigate the genetic regulation of arrested development in the bovine lungworm *Dictyocaulus viviparus* gene expression has been compared between third stage larvae, where inhibition had been induced by cooling at 4 °C for 6 weeks (L3-i), normal non-induced third stage larvae (L3-ni) and third stage larvae from a genetically changed, inhibition deprived laboratory strain (L3-id) by differential display reverse transcription polymerase chain reaction (DDRT-PCR).

After mRNA isolation reverse transcription has been performed using 3 anchor primers followed by PCR using 9 random primers for each cDNA pool. Amplified DNA from each L3 population and each primer combination was electrophoretically separated on a sequencing gel. Differentially expressed bands were isolated, reamplified and sequenced.

A total number of 35 cDNA bands were isolated that were exclusively expressed by inhibition induced larvae (L3-i) and not by L3-ni and L3-id. It is assumed that arrested development in *Dictyocaulus viviparus* is regulated via thermosensitive receptors that directly or indirectly activate one or a cascade of hypobiotic larvae constitutive genes. (The work was supported by a grant from the German Research Council, DFG grant No SCHN 267/9-1).



The red fox (*Vulpes vulpes*) as an indicator for the occurrence of liver flukes (Opisthorchiidae)

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Opisthorchiidosis is known from several Asian countries and parts of the former U.S.S.R. as a serious medical problem. Since in the past only single findings of *Opisthorchis felinus* were made in cats, foxes and otters, and no case of human opisthorchiidosis had been reported for the last 10 years in the indigenous population it was believed that these flukes do not play a major role in Germany. After HERING-HAGENBECK & SCHUSTER (1996) described a local focus of Opisthorchiidosis in the Eastern part of Brandenburg State (Germany) with cats acting as final hosts, two species of *Bithynia* snails as first, and 6 species of cyprinid fish as second intermediate hosts, investigations had started to draw a fluke distribution map for this federal state and the city of Berlin. First data of this research are presented in this paper. Up to now, 700 red foxes shot in a rabies control programme were dissected. 267 (=38%) were positive for liver flukes. With *Metorchis bilis* as most frequently occurring species (prevalence: 34%) up to a maximum of 1,074 flukes per liver were found. *O. felinus* and *Pseudamphistomum truncatum* were present in 8% and 0.7% of foxes with maximum numbers of 169 and 78 flukes respectively. While *O. felinus* was mainly isolated from the bile ducts of the liver *M. bilis* was more often found in the gall bladder. Only in cases of a high infection intensity higher numbers of *M. bilis* were found in the bile ducts. A marked proliferative cholangitis and pericholangitis even in livers containing moderate numbers of flukes was the main pathological finding.

Due to the high prevalences found in the current investigations the red fox can be regarded as a good indicator for the occurrence of these flukes. It also can be concluded that an infection risk exists for those people who include the raw or unwell cooked cyprinid fish into their diet.

Reference:

HERING-HAGENBECK, S., SCHUSTER, R. (1996): A focus of opisthorchiidosis in Germany.- Appl. Parasitol. 37: 260 - 265.

Antibody-secreting cell probes versus whole serum probes for antigen identification in *Haemonchus contortus*

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In vivo induced probes generated from antibody secreting-cells (ASC) of lymph nodes draining an infectious site have been found to be much more restricted in terms of their antibody profiles when compared to serum probes. Antigens identified by ASC probes in a variety of pathogenic agents have been found to be stage-specific and thereby fulfill a major criterium for candidate vaccine antigens. Three 3-month-old Merino sheep were continuously infected on a weekly basis with low doses of 3rd stage infective larvae (L₃) of *Haemonchus contortus* until faecal egg counts became negative (immune sheep). One month after the last infection two of the three sheep were challenged with 200000 L₃. Lymphocyte culture supernatants (ASC probes) were generated from the abomasal lymph nodes of challenged and unchallenged (negative controls) immune sheep. Western blots of whole somatic *H. contortus* L3 extract were probed with the different supernatants as well as with pre- and post-challenge sera derived from jugular blood samples. The number of antigens identified by ASC probes proved to be much more restricted when compared to pre- and post-challenge sera. Only the ASC probes of the challenged sheep recognized four L₃ antigens of approximately 197 kDa, 94 kDa, 60-80 kDa and 47 kDa. Besides the fact that a complex range of antigens was identified by using both pre- and post-challenge sera of the same animals, no differences in the antigens detected were observed between the two serum probes.

The determination of serum pepsinogen as an indicator for parasitic gastritis in cattle: Sense or nonsense?

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Ostertagiosis is one of the major factors influencing weight gain in cattle in their first grazing season. Since Ostertagiosis often occurs in combination with other trichostrongylids of minor importance, and thus, the interpretation of e.p.g. values may lead to false conclusions. Measuring blood pepsinogen levels was suggested as a parameter to forecast abomasal worm burdens in cattle. In our own investigations two cattle herds consisting of 40 and 35 heifers respectively, were under study during their first grazing season on pastures located in the Northern part of Germany where ostertagiosis occurred in previous years. Fecal and blood serum samples were collected at the time cattle are sent to pasture and at subsequent 14 d intervals. All animals were necropsied between 14 and 18 weeks after turn out and examined for trichostrongylids of the digestive tract within 14 and 18 weeks after turn out. First trichostrongylid eggs appeared already at day 28 in 50% of the cattle examined and reached maximum values of 433 and 600 e.p.g. respectively in average 7 weeks after turn out when both herds had to be treated due to a clinical outbreak of dictyocaulosis. A second peak in e.p.g. appeared with mean value of 500 e.p.g. 6 weeks after treatment. While levels of serum pepsinogen (s.p.) almost remained stable in one herd over the entire pasture period a considerable increase of s.p. was observed in the other herd 10 weeks after treatment. *Ostertagia ostertagi* burdens ranging between 500 and 118,000 adults were found at necropsy. A stratification depending on the s.p. levels at necropsy demonstrated that the highest average *Ostertagia* burdens were found in those groups of cattle showing the highest s.p. levels whereas the average number of *Cooperia oncophora* was equal in each group. Since the classical determination of s.p. levels is a time and cost intensive procedure its practical value as an early indicator for ostertagiosis remains questionable.

Gastrointestinal nematode infections in first-grazing season calves in Western Europe and the role of chemoprophylaxis

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Research on the prevention of gastrointestinal nematode infections of calves has concentrated on comparing a specific chemoprophylactic treatment to an untreated control group on particular farms. Here, the results from the analysis of 85 studies of first-grazing season (FGS) calves from 13 countries in Western Europe over a 26 year period are presented. Control and chemo-prophylactic treated calves were considered, with all chemoprophylaxis (slow- and pulse-release boli, strategic treatments) applied early in the FGS. Two infection levels emerged, characterized by whether or not control groups had an outbreak of parasitic gastro-enteritis (PGE) - 'clinical' (53 studies), and 'sub-clinical' (32 studies). Younger calf groups were significantly associated with PGE outbreaks. Mean faecal egg counts in 'clinical' controls were significantly higher than 'sub-clinical' controls during the FGS, and overall peaked at 275 and 100 EPG, respectively. Maximum pasture larval counts were also significantly higher on 'clinical' pastures, with 44% of pastures >10000 L₃ kg⁻¹ dry herbage by the end of the FGS, but only 15% of 'sub-clinical' pastures. There was a significant positive relationship between log transformed worm burdens from tracers put onto pasture for two weeks and the corresponding pasture larval counts. Weight gains in the 'clinical' controls (375g/day) were significantly lower than 'sub-clinical' controls (530g/day). PGE was not observed in any of the chemoprophylactic treated groups, but in studies with 'clinical' controls, treated groups had significantly higher faecal egg and pasture larval counts compared to treated groups with 'sub-clinical' controls. Better weight gains were significantly associated with the duration of the chemoprophylactic systems used. However, weight gains in chemoprophylactic treated groups in 'clinical' studies (600g/day) were significantly lower than treated groups in 'sub-clinical' studies (690g/day), and were not significantly different from 'sub-clinical' controls. That is, on heavily infected pastures chemoprophylaxis prevented PGE, but calves suffered production losses.

Diagnosis of Cowdriosis using monoclonal antibody to a conserved *Cowdria ruminantium* surface antigen

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To characterize *Cowdria ruminantium* elementary body (EB) antigens which can be used in diagnostic and immunization studies, monoclonal antibodies were developed from mice immunized with purified EBs of the Crystal Spring strain. Monoclonal antibody 446.15 of IgM isotype reacted with the surface of EBs both in acetone fixed and in live IFA tests. On Western blots, the mAbs reacted with a major *Cowdria* antigen of approximately 68 kDa and two lower molecular weight antigens of approximately 40 and 23 kDa. The mAb did not crossreact with *Erlichia canis* antigens loaded at 40 µg/well. To demonstrate that the antigens recognized by the mAb were encoded by the parasite, EBs were metabolically labelled in culture. On immunoprecipitation of ³⁵S-methionine labelled antigens, mAbs 446.15 immunoprecipitated two major antigens of approximately 80 and 30 kDa. Several other weakly labelled proteins including 68 and 40 kDa antigens were immunoprecipitated. No labelled antigens were immunoprecipitated by an isotype control mAb WM25. Silver staining of antigens from a mAb 446.15 affinity chromatography column indicated that a major antigen of 68 kDa and minor antigens of 40 and 23 kDa were purified. To determine the location of the antigens on EBs, immunogold labelling and electron microscopy tests indicated that mAb 446.15 recognized antigens on the surface of elementary bodies. To determine the diagnostic potential of the mAbs, purified and sonicated EBs were coated onto ELISA plates and sera samples from heartwater endemic regions in Kenya and Zimbabwe tested in a competitive ELISA format.

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The Critical Test for *Anoplocephala perfoliata* in equids and efficacy of B1995 oral paste for the parasite

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Eighteen equids were given an oral paste containing B1995 at 0.5, 1.0, 1.5 or 2.0 mg B1995/kg body-weight, euthanised 48 hr later and examined for tapeworms. After treatment, all feces from each equid was examined for tapeworms. Three untreated equids were necropsied to determine normal position of tapeworms in the intestine. Only worms with scolices were counted in assessing efficacy and were deemed abnormal if disintegrating or had a brown discoloration. At necropsy, worms were noted as attached to or detached from the intestine.

In untreated equids 1040 normal worms were in the cecum with 74 detached. One detached worm was in the ventral colon. The percentage of detached worms in the 3 equids ranged from 4.1 to 10.7. In 18 treated equids 61 worms were recovered; 9 attached to the cecum and others free in the intestine. All attached worms and 5 detached (cecum and ventral colon) were normal. All other detached worms including 2 in the cecum were abnormal. There were 198 abnormal tapeworms in the feces. In all but 2 of the 18 treated equids the efficacy of B1995 was 100%; in one treated at 1.0 mg/kg efficacy was 96.2%, another at 0.5 mg/kg was 38.1%.

A Critical Test with a 24-hr post-treatment period, no examination of feces and worms classified as removed by treatment if found distal to the cecum has been recommended (Vet Med 81:280, 1986). If in this trial those procedures were followed, the number of tapeworms in the animals at the time of treatment and possibly efficacy of the drug would have been underestimated. The 2 modifications of the Critical Test will be compared.

Diagnostic tests in experimental and natural *Trypanosoma evansi* infection

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Trypanosoma evansi causes 'Surra', an economically important disease responsible for high morbidity and mortality in Indian livestock. An improvement in the diagnostic approach is based on the detection of circulating antigens in the sera of experimental and natural cases of trypanosomiasis.

Detection of circulating antigens and antibodies of *T. evansi* in sera of experimentally infected rabbits with different stocks of *T. evansi* by ELISA and comparison of its efficacy with counter immuno-electrophoresis (CIE) in sera of naturally infected animals like horses, buffaloes and camels was carried out.

Diagnosis by serological tests was earlier than detection by parasitological (wet blood examination) in experimental infections of rabbits. Antigen-detection tests became positive earlier than the antibody-detection ELISA and could not detect infection as early as 24 hour post-incubation with range of 1-2 days post-inoculation (DPI), while CIE became positive after two DPI. Earliest positivity by Ab-ELISA was 4th day with range of 5-8 days.

In the diagnosis of naturally infected/suspected cases among various animal species also, the detection by serological tests was higher than the parasitological examination. The Ag-ELISA proved most sensitive and the correlation between antigenemia and parasitaemia was better than that between antibody and parasitaemia. The potential of Ag-ELISA as a diagnostic tool for epidemiological studies on trypanosomiasis was confirmed.

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Vaccination against abomasal nematodes - giving the worms a taste of their own medicine?

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Haemonchus galactose containing glycoprotein (H-gal-GP) is an integral membrane protein complex on the luminal surface of the gut of *H. contortus*. It is highly protective for lambs when used as an immunogen against experimental challenge. The antigen resolves into four major polypeptides by SDS-PAGE under non-reducing conditions, but numerous attempts by a variety of chromatography techniques to separate these components under native conditions have not proved successful, which is why it is thought to exist as a complex. H-gal-GP possesses two types of protease activity and the corresponding genes, encoding metallo-endopeptidase and pepsin-like enzymes, have been cloned and expressed. It seems reasonable to assume that these proteases digest the blood meal and that parasite digestion in sheep immunised with H-gal-GP is impaired by specific antibodies, leading to malnutrition, reduced fecundity, weakness and ultimately loss of worms. An equivalent of H-gal-GP is present in *O. ostertagi* and *O. circumcincta* and the latter has been found partially protective for sheep. If inhibition of nematode pepsin is indeed the mechanism of vaccine immunity against *O. circumcincta*, the tables will have been well and truly turned!

Descriptions of shape and size variability in *Eimeria* oocysts by use of digital image analysis and Fourier transform

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This study describes shape and size variability in frequency (as quantitative data) and in spatial (as contour diagrams) domain by characterisation, classification, identification and reconstruction of bovine *Eimeria* spp.

The morphology of 810 *Eimeria* specimens was defined in binary (b/w) digital images by pixels of their oocyst outline. A Fourier transform of pixel positions yielded uncorrelated and ordered size and shape features. To classify coccidia, the quantitative data were employed in an agglomerative clustering by average linkage algorithm. Size and shape were assigned equal weight.

An inverse Fourier transform served to reconstruct oocyst outlines, i.e. outlines of average shape and size, from mean values of features in resulting clusters. Clusters were subsequently identified based on their average morphology by comparison with drawings in an earlier taxonomical work. Identification was done by eye, because use of minimum Euclidean distances proved erroneous.

Given normal distribution of features, 500 oocyst outlines were simulated for each cluster representing a species. Shape/size variability was presented in contour diagrams.

The study provides rigorous descriptions of shape/size variability based only on *a priori* knowledge of a 'typical' specimen for each identified group (*Eimeria* spp. in an earlier taxonomical work).

Host genetic resistance to ticks and tick-borne diseases

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Ticks are an important constraint on the livestock industry, particularly in tropical and subtropical areas, mainly because of the diseases they transmit and the cost of control. Conventional control is by acaricides whose cost and the development of resistance by target populations are major drawbacks in their intensive use. A realistic alternative is to make strategic use of the hosts' innate and acquired ability to develop protective immunity against ticks and tick-borne diseases, especially in areas where extensive livestock ranching is practiced. The principle of host resistance to one-host ticks has been extensively documented and been adequately proven in the case of multi-host ticks for Zebu and crossbred cattle. The hereditary nature of host immunity has been adequately established to merit its selection and institute control strategies based on host resistance. The expression of host immunity has been shown to result from a wide variety of humoral, cellular and both immediate and delayed hypersensitivity responses. The application of the concept in livestock production systems is hampered by the potential economic implications to the producer not being accurately assessed, negative producer attitudes and its application by producers not being seriously motivated.

Variation in the mitochondrial NADH dehydrogenase I gene between different geographical isolates of *Schistosoma japonicum* detected by sequence- and restriction enzyme analysis.

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We here report the sequence of the mitochondrial NADH dehydrogenase I gene of nine different geographical isolates of *S. japonicum*. Six of the isolates were collected from six different provinces of P.R. China, while three isolates were laboratory maintained isolates. The three laboratory isolates originated from Anhui and Chekiang in P.R. China and Irosin in the Philippines.

Total DNA from pooled male and female worms of each isolate was extracted by the QiagenTM Genomic Extraction Kit. The extracted DNA was amplified employing a standard PCR technique using forward (5'AGATTC-GTAAGGGGCTAATA3') and reverse (5'ACCACTAATAATTCACCT-TTC3') primers and the resulting 475 bp gene fragment was sequenced. Based on the sequencing data, the amplification product was digested using the restriction enzyme *Rsa*I. The amplification and digestion were repeated on 30 individual worms (15 males and 15 females) of each of the three laboratory isolates.

The results of the sequencing showed a high degree of homology (98-99.7%) between the isolates. A single basepair mutation was detected between the Anhui isolate and the two other laboratory isolates resulting in a restriction fragment length polymorphism (RFLP). This RFLP was observed in all the analysed individual worms.

The described technique has proved to be an excellent tool to distinguish between the two isolates of *S. japonicum* maintained in our laboratory. This approach will be used in future experiments to follow the fate of specific *S. japonicum* subpopulations, or cohorts, in trickle infected pigs.

Presentation of an interactive, multimedia training program to teach helminthology in dogs and cats

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The influence of computer-based training is constantly increasing in the veterinary medical education. The advantages of interactive multimedia programs, such as immediate feedback, accommodation of individual learning styles, non-linearity and media variety have been well demonstrated. The objective of this study was to develop a computer-based training (CBT) program on „Round- and tapeworms in dogs and cats“ for veterinary students, laboratory technicians and veterinarians. The interactive multimedia program trains basic parasitologic knowledge. After working with the program the user should understand basic concepts about parasitology, diagnostic tools and therapeutic strategies. Challenges lay in the transformation of linear structured, conventional media such as textbooks and videos into a non-linear structure of an interactive multimedia program. The subject matter was analysed in respect to structure and content and presented in four chapters (basics, diagnostics, therapeutic concepts, zoonoses). Adequate media were selected and enhanced or produced to illustrate each aspect. While text was used sparsely, still images, animation, video and sound clips were heavily utilised to make the program attractive and to improve knowledge retention. Interaction with the user and prompt feedback were used to ensure a high educational value. The program was developed to facilitate learning by the structure and layout of the different topics. The program was written in a multimedia authoring language (Multimedia ToolBook 3.0 ©) and distributed on CD-ROM. An interdisciplinary team consisting of a screen designer, an information scientist and a veterinarian assured an intuitive navigation, a user-friendly screen design, an effective didactic approach and a high level of student's motivation. The program will be evaluated with third year students and veterinarians in respect to effectiveness and users' perceptions in July 1997.

Effect of pre-immunisation with *Psoroptes ovis* extracts on experimental mange.

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Scroptic mange is an ectoparasitic disease produced by the mite *P. ovis* that causes a severe exudative dermatitis. Its lesions, that may cover the whole body surface, are characterised by scabs and intensive itching. Sheep that have suffered mange show less severe clinical signs when re-infected with *P. ovis*. In order to establish if whole *P. ovis* antigens may induce this protection, *P. ovis* extracts were prepared and injected to naive sheep. For total extracts (TE), 3 g of pooled *P. ovis* were homogenised in 1 ml of buffered saline and centrifuged 10 min at 250 x g. For soluble extracts (ST), the supernatant was centrifuged 20 min at 12,000 x g. In both instances, 1 g of protein/sheep was injected to 8 sheep in complete Freund's adjuvant. Sheep were re-injected 15 and 30 days later with the same extracts in incomplete Freund's adjuvant. Seven control sheep were injected at the same times with buffered saline in adjuvants. All sheep were challenged 45 days later with 100 mites/sheep in the shoulder area. Animals were evaluated weekly during 2 months, by calculating the rate of lesions/body surface and the number of mites/cm², and results were compared by Split Plot and Variance analysis. Whenever lesions covered 70% of the body surface, that sheep was removed from the experiment and treated.

The difference between control and SE injected sheep were significant both in rate of lesions (means: SE = 24 ± 10 %; control = 55 ± 19 %; p < 0.01) and in the amount of mites/5 cm² (p < 0.01). Although TE injected animals had less severe lesions than control, this difference was not significant. All control animals had to be treated by week 7; none of the SE or TE injected animals had to be treated before the end of the experiment. Our results show that *P. ovis* soluble extracts may induce protection against mange in sheep. These data may be useful for the development experimental vaccines.

Neospora caninum antibody activities during bovine pregnancy

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The cyst-forming coccidian parasite *Neospora caninum* belongs to the family Sarcocystidae of the phylum Apicomplexa. *N. caninum* is recognised as the major cause of infectious abortion in cattle in California, and has been reported as a cause of abortion in many other countries. The only known route of infection is the congenital, and *N. caninum* can be transmitted from a cow to its progeny at consecutive pregnancies.

In this study, antibody activities were measured in blood samples collected monthly during two pregnancies from 13 heifers. The heifers were born between July and September 1993. They were all from a herd of Swedish Red and White breed dairy cattle in which *N. caninum* had previously been isolated from a stillborn calf. Two of the heifers aborted in their first pregnancy at 5 and 8 months of gestation, respectively. Blood samples from all heifers were collected from one month before the first insemination until one month after the second parturition. The blood was analysed for presence of antibodies to *N. caninum* with an iscom ELISA. The cut off absorbance in this test was 0.20 absorbance units. In the blood samples collected before the first insemination the absorbance values were between 0.50 and 1.10 for all animals. During the observation period they were never below 0.40 absorbance units. At mid-gestation the absorbances rose and these higher absorbances were maintained until calving or one month after calving for most of the animals.

a rise of
2 titre steps
at 4 months

Long-Lasting Parasite Control in the USA Utilizing IVOMEC® (ivermectin) SR Bolus

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The IVOMEC SR Bolus is a novel formulation of ivermectin designed to deliver 12 mg of ivermectin per day for 135 days. This formulation provides a high degree of efficacy against existing infections of gastrointestinal roundworms, lungworms, mange mites, sucking lice, cattle grubs and certain tick species. In addition, it provides grazing cattle with 135 days of protection from reinfection with gastrointestinal roundworms and lungworms, as well as certain arthropod parasites. This high level of parasite control prevents clinical disease due to gastrointestinal nematodes, lungworms, and external parasites and minimizes productivity losses associated with these parasites. Ten field studies comparing performance of treated stocker cattle against untreated controls demonstrated statistically significant (p < 0.01 to 0.05) average weight gain advantages ranging from 41 to 111 pounds. Five trials conducted in the northern USA and in Canada also showed that treated dairy replacement heifers had a statistically significant (p < 0.05) average weight gain increase of 32 pounds when compared with untreated controls. One grazing to feedlot study performed in stocker beef calves showed a 54.8 lb. weight gain difference (p < 0.05) between IVOMEC SR Bolus treated calves and a group of calves treated twice with benzimidazole anthelmintics and ectoparasiticides. Details relative to the technology, efficacy and prophylactic effect against specific species of parasites and resulting productivity benefits will be briefly described.

Control of bovine fasciolosis associated with farming irrigated rice

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Recommendations for control are based on results of a four-year epidemiological study of fasciolosis in Indonesia, sponsored by the Australian Centre for International Agricultural Research. Observations were made in an area where the agricultural system is based on production of one or two crops of irrigated rice per year followed by a period of a few months when the land is left fallow or used to produce dry-land crops. Farmers commonly kept 1-3 Ongole cattle or water buffaloes which provided the main source of draught power. They survived on crop residues and herbage from areas not under crop. The main habitat of *Lymnaea rubiginosa*, the snail host of *F. gigantica* in Indonesia, was irrigated rice fields. They were introduced annually with irrigation water from the few dry-season refuges in springs and permanent streams. Snails with the highest prevalence of infection with *F. gigantica* were found in rice fields within about 50 metres of a cattle pen. Tracer studies revealed that over 80 % of infection occurred during the period when rice was being harvested (January to June). The main risk factors for infection of cattle were shown to be grazing, drinking, or eating fresh rice straw from recently harvested rice fields, especially those within about 50 metres of a cattle pen. Fresh rice straw should be exposed to sunlight for 3 day or dried in the shade for 4 weeks to kill metacercariae, before being used as fodder. An alternative is to feed only the top two-thirds of rice stalks as the metacercariae are located on the bottom third (below the level of water in the field in which the rice was previously growing). A single annual treatment with triclabendazole 6 weeks after harvest of the last of the second seasonal rice crop in an area gave good control but all cattle sharing common grazing must be treated, plus all introductions to the area throughout the year. Biological control using 5-10 ducks infected with *E. revolutum* was highly effective in reducing infection in snails in high-risk fields near cattle pens. This approach was based on observations that *L. rubiginosa* infected with *E. revolutum* are refractory to infection with *F. gigantica* and that an existing infection with *F. gigantica* in snails is aggressively displaced by subsequent infection with *E. revolutum*. It was necessary to locate the duck pen over the effluent drain from a cattle pen so that duck and bovine faeces entered the rice field together, or to mix duck and bovine faeces before using it as fertiliser in rice fields.

Improving the equine by parasitic control

E D Svendsen

The International Donkey Protection Trust, Sidmouth, Devon, UK

The International Donkey Protection Trust celebrates its 21st birthday this year. Its main achievements have been in improving the working conditions of mules and donkeys by the efficacious use of anthelmintics. Scientific studies in various parts of the world have proved beyond doubt that not only can anthelmintics increase the general health of the donkey, but increase its working life substantially, to the great benefit of its often impoverished owner.

The production of The Professional Handbook of the Donkey, with articles and papers from vets world-wide, has been given free of charge to any veterinary university requesting it, and has become the major teaching aid for veterinary students world-wide.

Special training schemes for overseas veterinarians are provided both at the Donkey Sanctuary, Sidmouth, Devon, U.K., and in co-operation with the Weipers Centre at the University of Glasgow Veterinary School.

The charity is working in Mexico, Ethiopia and Kenya, where either permanent or mobile clinics are in use, and working on smaller projects in Greece, Turks & Caicos Islands, Jamaica, Tanzania and Spain.

Efficacy of ivermectin and praziquantel against *Anoplocephala perfoliata* in horses

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Six naturally infected horses treated with a paste formulation at dose rate of 0.2mg of ivermectin and 2.5mg of praziquantel/kg b.w. was 100 % effective in the removal of *Anoplocephala perfoliata*. The number of cestodes found non-attached to the mucosae of cecum and colon varied from 3 to 136, with an average of 42.5 cestodes.

Resistance of *Oesophagostomum quadrispinulatum* trickle infected pigs to challenge infection

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It is generally considered that no immunity exists against *Oesophagostomum* spp. infections in pig. Only few reports exist concerning resistance to reinfections, especially in the case of *O. quadrispinulatum* infection. In the present study trickle infections were given to imitate more closely the natural situation. Two groups of pigs were trickle infected with 1000 *O. quadrispinulatum* larvae given via feed twice a week. After eight weeks of exposure all pigs, including non-infected controls, were treated with fendendazole. Ten days later all pigs received a single inoculation with 5000L₃, of either *O. dentatum* or *O. quadrispinulatum*, respectively. Faecal egg counts were determined throughout the study. Pigs were slaughtered six weeks after the challenge infection and worm burdens were determined. No differences were found in the length of prepatent periods between single (control) and challenge infection groups. All pigs started egg excretion 17-23 days after inoculation. Pigs trickle and challenge infected with *O. quadrispinulatum* had significantly lower worm burdens at autopsy than control and *O. dentatum* challenge infected pigs indicating some degree of host immunity in the case of homologous challenge infection but not in the heterologous infection.

Towards a tick control programme on a farm with Blue ticks showing resistance to amitraz, pyrethroids and chlorfenvinphos.

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A coastal farm in Kwa Zulu Natal had a history of *Boophilus decoloratus* and *B. microplus* resistance to chlorfenvinphos, pyrethroids and amitraz. The objective of the trial was to develop a control strategy using currently available chemicals. A combination product containing chlorfenvinphos and esfenvalerate followed by amitraz was most recently used by the farmer. Ticks had showed resistance to each of these chemicals in less than 12 months after their introduction.

Larval and adult immersion tests, as well as small scale hand spraying showed chlorfenvinphos to be less resisted than cyhalothrin or amitraz.

A pasture spelling plan was introduced, the herd injected with doramectin 10 mg/ 50 kg and dipping weekly in chlorfenvinphos started. Tick control was monitored by collecting Blue tick scrapings monthly, as well as recording the clinical infestation rate in the herd. Samples of dipwash were collected weekly and analysed for chlorfenvinphos content.

The initial infestation rate of 70% declined to 0% by day 14. This remained at levels below 2% for 22 months. Skin scrapings showed adult engorgement to have stopped after 1 month and nymphal engorgement after 2 months. There were no ticks recovered from scrapings taken during the winter period in the second year of dipping. After 22 months, resistance was again apparent and a change of dipping material necessitated.

The system allowed chlorfenvinphos to be used for 22 months, whereas previously resistance had resurged within 12 months. In this trial, larval resistance tests alone could have been used to indicate the most suitable chemical to be used in the diptank.

Epidemiology of cattle strongyle in France-Results of herbal sampling in eight different areas.

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Herbal samples have been taken in France from eight different areas grazed by young cattle between June and October 1996 in order to count the infective gastrointestinal and respiratory strongyle larvae. The arithmetic means (L3 per kg herb) of the different species are : 3652 *Cooperia* spp (67,8%), 1466 *Ostertagia* spp (27,2%), 220 *Nematodirus* spp (4,1%), 33 *Trichostrongylus* spp (0,6%) and 14 *Dictyocaulus viviparus* (0,3%).

The results show a significant prevalence of *Cooperia* larvae throughout the season and a postponed summer rise related to the drought. The higher numbers of larvae have been counted at the end of September. Usually, under the temperate climate of France, the summer rise is earlier for the north and at that time for the south. The prevalence of *Cooperia* spp has to be stressed to plan specific treatment when calves are weaned during late summer.

For the last few years, the pathogenic effect of *Cooperia* has been increasingly taken into account. These worms alone or associated with *Ostertagia* are now a severe threat for young cattle in temperate areas. As these parasites are quickly expelled as soon as the immune reaction is developed, necropsies at the end of grazing season are often not informative. Furthermore, peptic digestion of intestinal mucosae for recovery of larvae is not usually performed. It appears information on *Cooperia* spp has to be assessed to improve the production of calves at pasture.

The molecular epidemiology of infections with *Giardia* and *Cryptosporidium*

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The importance of zoonotic transmission in the epidemiology of both giardiasis and cryptosporidiosis has still to be determined. In both cases, animal reservoirs appear to play a role in disease outbreaks but identifying sources of infection at a local level has proved very difficult using conventional techniques. The aetiological agents of giardiasis and cryptosporidiosis in humans and other mammals are morphologically uniform yet exhibit considerable genetic heterogeneity. Genetic characterisation of parasite material has, until recently, required laboratory amplification of isolates collected in the field or from clinical specimens, which has severely limited attempts to determine transmission patterns at a local level. In our laboratory, we have developed a range of molecular epidemiological tools based on the polymerase chain reaction (PCR) for characterising isolates of *Giardia* and *Cryptosporidium* directly from both faecal and environmental samples. We have applied these tools for characterising isolates of *Giardia* and *Cryptosporidium* from companion animals and livestock and have found evidence for both host-adapted and zoonotic cycles of transmission.

Trichinella ssp. in wild red fox (*Vulpes vulpes*) in Norway

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Trichinellosis in wild carnivores is quite common in Norway, and in routine investigations of material submitted to the National Veterinary Institute during the period 1960-1991, the prevalence was found to be 25% in the red fox, but much lower in badger (*Meles meles*), mink (*Mustela vison*), marten *Martes martes* and wolverine (*Gulo gulo*). Investigations in the arctic region (Svalbard) has also shown a high percentage of *Trichinella*-infected polar bears (*Thalarctos maritimus*) and Arctic blue fox (*Alopex lagopus spitzbergensis*). Occasionally, trichinellosis has been found in pigs at the obligatory slaughterhouse control, the two last cases were seen in 1994.

The trichinellas found in mainland Norway were always assumed to be *T. spiralis*. In 1989, freeze resistant trichinellas were detected in farmed Arctic blue fox in northern mainland Norway, and they were identified as *T. nativa*. It was still believed, though, that this species was restricted to the northernmost part of the country.

During the winter season of 1994-95, 65 red foxes from various parts of Norway were examined for ecto- and endo-parasites. Trichinellas were detected in only four of these, which is a dramatic reduction from previous investigations. The parasites were sent to Dr. E. Pozio at the Trichinella Reference Centre in Rome for further identification. From three of these *T. nativa* were identified, and from one *T. britovi*. They were all from the southern part of Norway. There was no finding of *T. spiralis*.

The number of foxes in Norway has declined dramatically since *Sarcoptes scabiei* was introduced to the wild fox population in Norway in the mid-seventies. The apparent decline in the prevalence of trichinellosis in Norwegian wild foxes might have been caused by this parasite, which more or less decimated the fox population in many areas. The material is very limited, but it may be speculated if only *T. nativa* and *T. britovi* are present in this country, and not *T. spiralis*.

Efficacy of eprinomectin against *Sarcoptes scabiei* infestation in cattle

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Four controlled studies and two field demonstration studies were conducted to determine the efficacy of a topical formulation of eprinomectin to control *Sarcoptes scabiei* infestation in cattle. The controlled studies, two in Europe using induced infestations in 3 - 6 month old animals, and two in the USA using natural infestation in adult dairy cows, were of similar design. In each trial, between 14 and 20 animals held in individual stanchions or stalls were randomly allocated to treatment once with eprinomectin topically at 500mcg/kg or with unmedicated vehicle. Assessment of *Sarcoptes scabiei* infestations was conducted using skin scrapings and counts of live mites before treatment and thereafter each 7 days to Day 28 (2 studies) or Day 56 (2 studies). In the field demonstration studies, 2 whole herds of *Sarcoptes* infested lactating dairy cows (59 in one herd and 80 in the other) were treated while held in the dairy parlour. The response to treatment was assessed clinically after 3 weeks and selected animals were assessed by skin scrapings for evidence of live mites.

In all 6 trials, excellent efficacy against *Sarcoptes scabiei* was demonstrated. In the controlled studies, 25 of 32 untreated animals retained *Sarcoptes* infestation at the final assessment. In contrast, eprinomectin caused a rapid reduction of mite numbers in treated animals. Low numbers were found on Day 7 and thereafter efficacy was > 99 % from Day 14 and 100 % at the final assessment on all 4 studies. In the field demonstration studies, the lactating cows responded to the treatment well. No live mites were found at the 21 day assessment and there was a marked clinical improvement in both herds. There were no adverse reactions to treatment in any of the 6 studies.

The data demonstrate that eprinomectin is an effective treatment for *Sarcoptes* infested cattle. The pour-on formulation and zero milk withdrawal make it a convenient treatment, particularly for lactating dairy cattle.



Prevalence of antibodies to *Cowdria ruminantium* from cattle on communal grazing in the North West Province of South Africa

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Heartwater (*Cowdria ruminantium*) is an important tick-borne disease of ruminants. In South Africa, little data is available on the serological status of cattle kept on communal grazing.

Rietgat and Madinyane are communal grazing areas in the North West Province, South Africa. Young cattle, 6 - 18 months old, and representative of the population, were bled each winter - 1991, 1992 and 1993. Anti-bodies to *C. ruminantium* were detected with the indirect ELISA(*) and/or IFA (+) tests.

At Rietgat, the point prevalence in 1991, was initially high, $P = 0.3^*$ (8/27), $P = 1.0^+$ (9/9), and stayed high in 1992, $P = 0.77^*$ (30/37), and in 1993, $P = 0.94^*$ (17/18), $P = 1.0^+$ (4/4).

At Madinyane, similar high prevalence to heartwater was recorded in 1991, $P = 0.89^*$ (17/19), $P = 0.24^+$ (6/25), and in 1993, $P = 0.83^*$ (40/48) $P = 1.00^+$ (25/25).

Both communal grazing areas had *Amblyomma hebraeum* present on the cattle and goats throughout the survey. Clinical cases of Heartwater were seldom recorded, and endemic stability to the disease was suspected.

Assessment of *Cooperia oncophora* side resistance between ivermectin, doramectin and moxidectin in cattle.

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A trial was conducted to evaluate efficacy of commercially available macrocyclic lactone endectocides against a strain of *Cooperia oncophora*, shown to be tolerant to ivermectin. Thirty-six healthy Hereford/Friesian cross male calves were used. On Day -21 each animal was infected with approximately 10,000 infective third stage larvae of *Cooperia oncophora* (Massey 95A - NZ 9-94) supplied by Drs. Charleston and Pomroy, Massey University, New Zealand, and passaged once at the trial site. Equal numbers of animals were allocated by restricted randomisation based on bodyweight to one of six treatments: Unmedicated control; ivermectin s.c. 200 mcg/kg; ivermectin pour-on 500 mcg/kg; doramectin s.c. 200 mcg/kg; moxidectin s.c. 200 mcg/kg; and moxidectin pour-on 500 mcg/kg. The calves were treated at the recommended dose rate on Day 0 and were euthanased by replicate on Days 14 and 15 for nematode parasite recovery. Animals treated with ivermectin pour-on or moxidectin pour-on had significantly ($p < 0.05$) fewer adult *C. oncophora* than the unmedicated controls. Efficacies for these groups relative to the control means were 91.6% and 91.4%, respectively. None of the treatment comparisons involving the injectable products were significantly ($p > 0.05$) different from the controls. Efficacy for these groups ranged from 27.9% to 57.0%. The reduced efficacy of the test compounds, particularly when administered by injection, against this 'Massey' strain of *Cooperia oncophora* indicates that side resistance occurs between ivermectin, doramectin and moxidectin. The data shows that topically applied macrocyclic lactones were more effective than those administered by injection at the recommended doses.

The use of Ivermectin for the control of strongyle species in donkeys; *Equus asinus*, using the egg reappearance period (ERP) as the parameter for assessment of efficacy.

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Modern day anthelmintic programmes for the control of endoparasites in equines is based on the use of broad spectrum anthelmintics every 4 - 8 weeks - a regime encouraging the development of resistance.

Research was undertaken to investigate the possibility of slowing down the development of resistance to anthelmintics and the possible reduction in the incidence of cyathostomiasis using faecal ERP as the criteria for periodic anthelmintic dosing.

This trial was conducted at four of the Donkey Sanctuary farms in Devon. The herds varied in age, population densities, pasture management and parasitological burdens. The donkeys were given Equisan (Ivermectin) at a dose rate $>200\mu\text{g/kg}$ prior to spring turnout.

The faecal egg count (EPG) was then monitored using the Modified McMaster technique by random sampling 15% of the herd. A total of 1521 donkeys were chosen using a randomisation programme and faecal sampling commenced eight weeks post worming and then fortnightly until the ERP reached the level of 25% of the herd having a faecal egg count of more than 200epg (Uhlinger, 1992).

The results of this trial show that with good pasture management the periodicity of dosing can be extended to allow anthelmintic dosing at spring turnout and prior to winter housing (up to 22 weeks) by closely monitoring the ERP.

This regime not only reduces the onset of anthelmintic resistance but the cost savings on purchase of anthelmintics exceed the increase costs of laboratory labour and materials producing a real financial benefit.

A preliminary survey on surra in Thai elephants (*Elephas maximus*)

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A preliminary survey on surra, which is endemic in Thailand, were studied in 115 captive elephants (*Elephas maximus*) from northeast Thailand during 1994-1995. The elephants' blood were examined for blood parasites by blood smear, microhaematocrit centrifuge test, buffy coat smear and mouse inoculation. The serum samples were tested for the antibodies against *Trypanosoma evansi* by enzyme linked immunosorbent assay (ELISA), card agglutination test for *T. evansi* (CATT) and indirect fluorescent antibody test (IFAT). The conjugate used in ELISA and IFAT were alkaline phosphatase rec-Protein G conjugate and FITC rec-Protein G conjugate, respectively.

It was found that all blood samples were negative for any blood parasites by blood smear, microhaematocrit centrifuge test and buffy coat smear. Forty-four of 115 blood samples were inoculated immediately into mouse and all were negative for trypanosome. By ELISA four serum samples gave the high OD values (OD value = 0.387-0.627), whereas, the average OD value of most elephants was 0.140 (0.106-0.233, SD = 0.021). Nevertheless it was noticed that the elephants, which gave negative results in ELISA, were also negative in IFAT and CATT. All positive samples in ELISA gave also positive results in IFAT. However, only two of four elephants with high OD values in ELISA, gave the positive results in CATT.

The efficacy of Endex^R on *Fasciola gigantica* and other gastro-intestinal nematodes in naturally infected cattle

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The efficacy of Endex^R, a mixture of 12 % triclabendazole and 7.5 % levamisole, on *Fasciola gigantica* and other gastro-intestinal (GI) nematodes in naturally infected cattle was studied under field condition. Forty-four cattle were selected and divided into 3 groups: Group 1 consisted of infected animals given a placebo, Group 2 infected animals given Endex^R 1mt/10kg and Group 3 animals free from parasites and given Endex^R 1mt/10kg. The numbers of *Fasciola* and GI nematode eggs per gram feces were counted before the experiment and during the study. Fluke burden of the cattle was determined by levels of serum g-glutamyltransferase (GGT) and alkaline phosphatase (AP).

In the treated cattle in Group 2 no *Fasciola* eggs were found in the feces from day 7 to week 12 of the experiment, whereas GI nematode eggs disappeared after day 7 to week 5 of the experiment. In contrast, in the untreated cattle in Group 1, parasite eggs were found from day 0 to the end of the study whilst the uninfected cattle in Group 3 were still free from *Fasciola* and GI nematodes egg. It was also noticed that on day 0 of the experiment there was no significant difference in the GGT value of cattle between Group 1 and 2. However, on week 4 after treatment the GGT value of cattle in Group 2 decreased and was significantly different from the GGT value of cattle in Group 1 ($p > 0.001$). The GGT value of cattle in Group 2 decreased to normal values on week 12, whereas the GGT value of cattle in Group 1 remained at a high level. There was no significant difference of the serum AP values of the cattle among Group 1, 2 and 3 and on day 0 and week 4.

A NOVEL KEY FOR IDENTIFYING NEMATODE INFECTIVE LARVAE (L3) FROM DOMESTICATED RUMINANTS

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Most keys for identifying L3 of nematodes from ruminants list measurement ranges together with other distinguishing features of the organisms. This makes it difficult, firstly, to notice salient relationships between L3 of the various genera, and thus to master the identification, except by experience, under the close guidance of an experienced person.

We have developed a simplified key which, in addition to the usual characteristics of shape (e.g. of the cranial extremity), expresses the lengths of the free tail sheaths (length from larval tip to end of the tail sheath) of the L3 in relation to one another, and not only as actual measurements. For instance, if the mean length of the tail sheath of *Trichostrongylus* spp. from sheep is assumed to equal x, then that of *Haemonchus* spp. is $\pm 2x$, *Oesophagostomum* $\pm 4x$ and *Nematodirus* $\pm 8x$, etc. Where applicable, additional characteristics are also used, for instance differences in staining with iodine, and the relative length of the filamentous portion of the tail sheath. For example, *Oesophagostomum* (sheath proportional length $\pm 4x$; filament $\pm 40\%$ of tail sheath) is differentiated from *Chabertia* (tail sheath $\pm 3x$; filament $\pm 25\%$) and *Gaigeria* (tail sheath similarly $\pm 3x$; filament $\pm 50\%$; oesophagus rhabditiform; uniform staining with iodine, etc.).

After some practice with the system (using a microscope familiar to the investigator) it is usually necessary to measure only a few sheath tails of the L3 in a given sample, whereafter most of the remaining L3 can be compared without having to be measured.

The host status of crested francolin for economically important ticks and a comparison of their burdens with free-living ticks on the vegetation

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The tick burdens of 30 crested francolin (*Francolinus saphaena saphaena*) shot at approximately 2-monthly intervals on two farms in the Northern Province namely, "Sandspruit", a mixed commercial beef cattle and game farm, and "Bison's Place", on which only game occurs, were determined. At the same 2-monthly intervals free-living ticks were collected from the vegetation on the farm "Sandspruit" by means of drag-sampling.

The birds harboured 5 tick species and their burdens were comprised of immature stages only. *Amblyomma hebraeum* was the most abundant and prevalent species, followed by *Hyalomma marginatum rufipes*. *A.hebraeum*, *Amblyomma marmoreum*, *Boophilus decoloratus*, *H.marginatum rufipes*, *Rhipicephalus appendiculatus*, *Rhipicephalus evertsi evertsi* and *Rhipicephalus zambeziensis* comprised the 8 species of ticks collected by drag-sampling the vegetation. *R. evertsi evertsi*, followed by *B. decoloratus*, was the most abundant. *A.hebraeum* and *R.zambeziensis* prefer woodland habitat to open grassland whereas *B. decoloratus* prefers open grassland.

The helminth parasites of the Laughing dove (*Streptopelia senegalensis*)

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Helminth parasites of Laughing doves were studied from June 1992 to October 1994. Faecal samples of more than 400 birds were examined. Necropsy experimentations indicated that cestodes were the most abundant helminth. The morphology of *Raillietina* spp. and *Hymenolepis* sp. were examined with the aid of light and scanning electron microscopy and salient results will be discussed.

Two nematode species were recovered from a few birds only; *Ascaridia columbae* from the small intestine and *Dispharynx* sp. from the pre-ventriculus.

The abundance and prevalence of these helminths will be discussed. The ecology of the hosts and modes of infection will be discussed in light of doves are mainly herbivorous and these cestodes and *Dispharynx* require intermediate hosts in their lifecycle.

Morphological aspects of an Ergasilid (Copepoda) from an Ariid catfish

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The family Ergasilidae (Copepoda) parasitizes a number of fish hosts ranging from mainly fresh water species to estuarine species. Members of the Ergasilidae are found in African estuarine environments on the gill filaments of hosts that belong to the family Mugilidae. However, one *Ergasilus* sp. was found on the white seacatfish, *Galeichthys feliceps*. A total number of six hosts was collected from the mouth of the Great Fish River, Eastern Cape. All were heavily infested with up to four specimens of *Ergasilus* sp. on a single gill filament.

In this paper the morphology of this species is described by means of light microscopy and scanning electron microscopy. Special emphasis is laid on the appendages of taxonomical importance, which include the antennule, antenna, oral appendages, prosome, pedigerous somites and urosome. The morphology of this species is also compared with its nearest congeners to establish its taxonomical status.

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In vitro techniques for evaluation different anthelmintic resistance status of the pig nodular worm *Oesophagostomum* spp.

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The objective of the present study was to evaluate the potential of three *in vitro* techniques for definition of anthelmintic resistance in *Oesophagostomum* spp. of pigs.

In this study anthelmintic susceptible or resistant isolates of *O. dentatum* and *O. quadrispinulatum* were used. Two isolates have had no prior exposure to anthelmintics and served as susceptible isolates. Six resistant or suspected to be resistant isolates have been isolated from pig farms with a known *in vivo* resistance status. Three isolates were artificially selected for resistance to anthelmintics. Two worm-free pigs were inoculated with approximately 8,000 L3 infective larvae and on days 25-45 post infection *in vitro* larval development assay (LDA), egg-hatch assay (EHA) and larval micromotility assay (LMA) were carried out.

For measurement of pyrantel and levamisole susceptibility the LDA was found to be able distinguish between susceptible and resistant isolates, but failed to show differences between ivermectin susceptible and resistant isolate. EHA was able to detect levamisole resistance, but did not show differences in response to pyrantel. LMA was not able to differentiate between anthelmintic resistant and susceptible isolates of *Oesophagostomum*. The LDA showed a greater difference in response between the isolates, better correlation with an *in vivo* data and seemed to measure the level of resistance accurately and more objectively compared to EHA and LMA. LDA has the potential to be useful in both field screening and research work. EHA and LMA have not been found suitable for detection of resistant worms, since the interpretation of the results is subjective and both assays have some limitations.

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Immunity development against *Mecistocirrus digitatus* (Nematoda: Trichostrongylidae) in calves

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The importance of the developmental stages of the abomasal nematode *Mecistocirrus digitatus* in inducing resistance to reinfection in calves was examined. Three groups (A, B, C) of calves were immunized for two consecutive days with a daily dose of 20000 *M. digitatus* infectious larvae. Group A was treated with ivermectin at 7 days after infection, Group B at 26 and Group C at 60 days after infection. Thirty days after treatment all animals were given a challenge infection of 30000 *M. digitatus* L₃. Three control animals were infected with 30000 L₃ only. All animals were necropsied 75 days after challenge. Prepatent period in the controls was 53 days; in immunized calves it averaged 60 days in Group A and more than 72 days in Groups B and C. Faecal egg outputs 75 days after the challenge infection were significantly lower in all three groups of immunized animals. Only adult worms were recovered from post mortems. Worm counts were similar in the controls and animals of Group A; in animals of Groups B and C more worms had matured than in the controls. Stunted growth was observed in worms obtained from immunized animals: male worms from Group B and females from Group C were significantly shorter than those from control animals. Following the challenge infection, the increase in serum pepsinogen levels was higher in immunized animals than in the controls. Based on the longer pre-patent period and the lower faecal egg counts, two infections with *M. digitatus* produced a significant immunogenic reaction in the host, most obviously after the fourth larval stage has been reached. However, there appears to be no obvious reason for the higher worm counts in the immunized animals.

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Prevalence and importance of helminth parasites in livestock of Zimbabwe

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Helminth infections that affect large numbers of animals are becoming economically more important. Nevertheless, they are largely neglected.

By coprologic examination of 64 482 cattle it was found that paramphistomes (56.8%), strongylid nematodes (47.6%) and *Fasciola gigantica* (36.8%) are the most prevalent parasites. *Schistosoma mattheei* (4.8%), *Moniezia benedeni* (1.6%), *Toxocara vitruolum* (1.3%) and *Trichuris globulosa* (1.1%) are less prevalent. *F. gigantica*, strongylids and premature paramphistomes are economically the most important internal parasites of cattle.

Coprologic and postmortem examination of 32 742 sheep and goats revealed that the commonest helminths are: strongylids (62.3%), *Moniezia expansa* (24.3%), paramphistomes (18.8%), *Stilesia hepatica* (18.1%), *Aviellina centripunctata* (9.7%), *T. globulosa* (7.9%) and *F. gigantica* (4.7%). *Haemonchus contortus* and *Oesophagostomum columbianum* are the main helminths of small ruminants.

Pigs are infected with *Ascaris suum* (7.9%), *Oesophagostomum dentatum* (3.6%) and *Trichuris suis* (4.9%).

Although anthelmintics have been used for a long time, the prevalence of parasitic infections remains the same, because control measures are not related to the existing husbandry practices, climatic conditions and available epidemiological data.

Cyathostome species in horses of Buenos Aires, Argentina. Their relative distribution

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Cyathostominae infect practically all horses through the world and may induce larval cyathostomiasis, a severe and often mortal syndrome. The pathogenicity of each of the many species of cyathostomes, or their combination, has not been studied. Although infection is practically 100 % prevalent in Argentina, no individual cases of larval cyathostomiasis have been reported. In contrast, we have reported 2 farms where 15 or more horses, i.e. all those of a certain age and condition, were simultaneously ill. The presence and relative distribution of cyathostome species was studied in horses from the Pampas region of Argentina. Their origins were: one of those horse farms where many horses had simultaneously suffered larval cyathostomiasis; a horse farm with no larval cyathostomiasis history; and abattoir viscera. Adult strongyles were recovered either from faeces after drug treatment, or from whole contents of necropsied large guts, and more than 1 200 individuals were classified following Lichtenfeld's chies. In all horses, *Cylicocycylus insigne* was the predominant species (above 37 % in all cases), followed by *C. nassatus*, *Cyathostomum catinatum* and *C. coronatum* or *Posteriosomum imparidentatum*. Other species found were: *Cylicocycylus ultrjectinus*, *C. calicatus*, *C. brevicapsulatus*, *C. auriculatus*, *C. radiatus*, *Cyathostomum tetracantum*, *C. labiatum*, *C. labratum*, *C. alveatum*, *C. pateratum*, *Cylicostephanus goldii*, *C. poculatus*, *C. minutus*, *C. longibursatus*, *Culicodonomophorus metanii*, but only in some horses and being less than 1 % representation. The distribution of species in both farms was different ($p < 0.02$) and differed from all other continents' published data. The species distribution found in colon and caecum also differed from published data: in caecum, there was 64 % of *Cyathostomum coronatum*, 21 % of *Cylicocycylus insigne*; 10 % of *Cylicostephanus poculatus* and 1 % of *Cylicostephanus calicatus*; and in colon: *Cylicocycylus insigne* 74 %; *Cylicocycylus nassatus* 23 %; *Cyathostomum catinatum* 1 %; *Cyathostomum coronatum* 1 %. This difference in species' presence, location and predominancy between the Pampas and other regions of the world may be related with the epidemiology of larval cyathostomiasis in the former region.

Persistent activity of doramectin injectable against *Ostertagia ostertagi* and *Cooperia oncophora*.

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Field observations suggest that duration of persistence is affected by infection level. The objective of the present trial was to evaluate the persistent efficacy of doramectin (SC) under conditions of different levels of daily infections mimicking overwintering (low), early mid season (moderate) and late summer (high) pasture infections (Europe). Thirty-six calves were divided into 6 groups. Animals of the C1 and T1 group received a daily infection of 200 L3 of *Ostertagia ostertagi* and 200 L3 *Cooperia oncophora*, animals of the C2 and T2 group received a daily infection of 1 000 L3 of each species and the C3 and T3 group received 10 000 L3 of each species. Animals of the T1, T2 and T3 groups were treated with 0.2 mg/kg doramectin (SC) on day 0. The infection period was 21 days for *Cooperia* and 28 days for *Ostertagia*. Animals were killed for worm counts five days after the last infection with *Ostertagia* (12 days after the last infection with *Cooperia*). The study demonstrated, at all infection levels, a persistent efficacy for doramectin of at least 28 days for *O. ostertagi* (>99 %) and at least 21 days for *C. oncophora* (>98%), the maximum measurable time-point in this experiment for the respective parasites. Therefore, no firm conclusions can be made on the effects of infection levels on duration of persistent efficacy, mainly because the duration of persistence of doramectin was longer than anticipated. A second study to determine the end-point of the prolonged efficacy is in progress.

Advances in the control of fasciolosis and schistosomosis

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Fasciolosis and schistosomosis in ruminants are of great veterinary significance in most parts of the world. Advances in the control of these cattle parasites have lagged behind the success which has been attained with control measures against other helminths and/or other infectious organisms. The factors which have contributed most to this relative lack of success are the limited number of new anthelmintics effective against flukes and the slow progress in vaccine development. Furthermore, in Australia *Fasciola hepatica* drug resistance to several anthelmintics has been demonstrated. However, recent studies suggest that the use of synergistic combinations of drugs of different chemical groups are successful in the treatment of fasciolosis even in resistant strains. Alternative recommendations for control of cattle fasciolosis based on grazing and fodder management and competition with other parasites for the snail host (e.g. *Echinostoma* from ducks), together with strategic treatment have shown to be effective in Indonesia. Concepts are now being developed to construct environmental risks assessment models for fasciolosis and schistosomosis by the use of (earlier) climate forecasts and soil hydrology geographic information systems.

Recent results also testify to the great potential of vaccination in controlling schistosomosis in cattle. However, it has been shown that the level of protection induced by vaccination may largely depend on the pattern of challenge, suggesting that a vaccine could only be of value in certain epidemiological conditions. Finally, recent investigations have suggested that studies using pigs as definitive host could aid research work aimed at developing new control strategies for *Schistosoma japonicum*.

Efficacy of doramectin pour-on in the control of nematode infections of grazing cattle

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A study was conducted to evaluate the efficacy of doramectin pour-on for the season-long control of gastrointestinal (GI) nematodes and lungworms in cattle. This study also examined the development of immunity against *Dictyocaulus viviparus* acquired during the grazing season in comparison with parasite naive calves (N group). Twenty principal animals were randomly allocated between a non-medicated group and a treated group. The animals in group D received doramectin pour-on (500 µg/kg) on days 0 and 56. The animals in group C did not receive treatment. Throughout the whole study, tracer calves were put on each pasture at three-week intervals to monitor the level of infestation. Treatment with doramectin pour-on at turn out and eight weeks later protected cattle from clinical signs of parasitic gastroenteritis and lungworm infection for the duration of the study (161 days), whereas the non-medicated group needed two intervention treatments against hush. Faecal egg counts and faecal larval counts reflected low infection levels of GI nematodes and lungworms in group D. Pasture larval contamination, as indicated by tracer worm burdens, was lower on the pasture grazed by the doramectin-treated group, compared with that of the control paddock. At turn-in, all the principal animals were treated with an anthelmintic. One month later, four animals from the C and D groups as well as animals of the N group received an oral challenge of 1000 infective larvae of *D. viviparus* and were necropsied 28 days later. The total numbers of lungworms recovered from animals of the C, D and N groups were 5, 47 and 264, respectively, indicating that treatment with doramectin pour-on did not adversely affect the development of immunity to *Dictyocaulus viviparus*.

Antibody responses in serum and abomasal mucosae of Nelore and Holstein calves infected with *Haemonchus placei*

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Haemonchosis ranks among the most important nematodiasis, being responsible for considerable economical losses worldwide. In this study the serum IgA, total IgG and its isotypes responses were observed, as well as the local response at the abomasal mucosae of cells showing a positive reaction from IgA antibodies against *Haemonchus placei* in cattle. Ten worm-free male calves (five Holstein-Friesian and five Nelore), nine months old, received a single oral infection with *H. placei*, at a dose of 1000 L3/kg bodyweight. Blood samples were collected before and after the infection for eight weeks, when at the end, animals were sacrificed. Worm counts of the lumen and mucosae of the abomasum were made, in several aliquots. Samples of the abomasal mucosae were taken for histochemical studies. The antibody response against adult *H. placei* antigen (10mg/ml) was measured by ELISA. Sera was diluted at 1:50 and used in duplicate. Peroxidase conjugated rabbit anti-bovine IgG (whole molecule) diluted 1:1000. Both Nelore and Holstein calves showed an increase of IgG antibodies against *H. placei* antigen 2 weeks after infection, peaking at the third week. Afterwards a similar and gradual decrease occurred until the 8th week. However, the absorbance values of Holstein calves tended to be higher than those from Nelore calves during the whole experimental period. Histological sections from abomasal mucosae were submitted to immunohistochemistry (avidin-biotin method) having as a primary antibody bovine anti-IgA. Positive and non-positive lymphocytes to IgA were quantified by means of a computerized analytical image system. Holstein-Friesian calves showed a larger number of positive lymphocytes to IgA than Nelore calves, in the upper area of the abomasal mucosae, eight weeks after infection. By this time, the parasitic burden of the Holsteins was significantly greater than that of the Nellores.

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The spread of porcine sarcoptic mange during the fattening period

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Using an indirect ELISA based on a whole body homogenate of *Sarcoptes scabiei* var *vulpes*, the development of serum antibodies to *S. scabiei* var *suis* during the fattening period was studied in two swine herds purchasing fatteners and performing age segregated production systems in Sweden. All the animals in one of the herds were treated with ivermectin during the first week after arrival, while no precautions were taken to prevent sarcoptic mange in the other herd.

In both herds, a transient immune response to *S. scabiei* was noticed in some animals during the early fattening period, reflecting that these pigs had been exposed to *S. scabiei* before delivery to the fattening units. It also indicated that they had been treated with acaricides close to allocation.

The incidence of animals seropositive to *S. scabiei* was initially low in both herds. The infection spread gradually as indicated by increasing optical densities of antibodies to *S. scabiei*, especially during the second half of the rearing period. The increase and spread of sarcoptic mange was found to be greater in the untreated herd.

The results showed that conventional pigs sold as fatteners at present may be infected with *S. scabiei* and that precautions aimed at preventing sarcoptic mange are therefore indicated in fattening herds. Although a single injection with the acaricide on arrival at the fattening farm decreased and delayed the spread of the infection, it did not eradicate the mite infection. The results also indicate that some piglet producing herds appear to be free from *S. scabiei*. Therefore, precautions aiming at preventing sarcoptic mange from occurring in fattening herds are warranted. The ELISA employed could be a valuable tool to discriminate between uninfected and *S. scabiei* var *suis* infected piglet producing herds.

Clinical signs and symptoms associated with donkey trypanosomosis

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The donkey is an important draft animal in most parts of Kenya where trypanosomosis is endemic in livestock. Little is known of the clinical presentation of donkey trypanosomosis. In a study to determine the epidemiological picture of donkey trypanosomosis in Lamu district, Kenya two visits were made to Lamu.

A total of two hundred donkeys were randomly selected. A thorough clinical examination was carried out on all the donkeys. The clinical signs were recorded and blood collected from clinically ill animals. The packed cell volume (PCV) was determined and presence of trypanosomes detected using the buffy coat examination (BCE). Presence of trypanosome antigens and anti-trypanosome antibodies in serum was determined in the laboratory using the ELISA technique. The presence of parasites in the buffy coat and antigens in the serum were regarded as active infection.

The frequency of different clinical signs in the animals were noted. In the donkeys with parasitaemia detected by the BCE, anaemia (PCV < 25 %) was the most frequent sign (70 %). Enlarged lymphnodes were observed in 45 % while skin lesions and a poor body condition both had a frequency of 36 %. Of all the animals with antigenaemia, which included all those with parasitaemia 48 % had skin lesions, 40 % were in poor body condition, 34 % had anaemia and 22 % had enlarged lymphnodes. Other signs observed at a lower frequency include respiratory distress and ocular lesions.

From these findings it was concluded that trypanosomosis does cause clinical disease in donkeys. In heavy infections, anaemia accompanied by enlarged lymphnodes, a poor body condition and various skin lesions are the most consistent signs. In less severe infections, the same signs appear but the study will be useful as a guideline to clinical diagnosis of donkey trypanosomosis.

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Epidemiology of ruminant helminthoses in Kakamega District : A sub-humid warm agro-climatic zone in Kenya

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A study was carried out on the epidemiology of helminthoses in cattle, sheep and goats in western Kenya, in a sub-humid fairly warm region. A total of 44 small holder farms comprising 245 cattle, 180 sheep and 54 goats were monitored monthly for faecal egg counts (FEC) and differential larval counts. Tracer sheep were used to monitor larval availability on pasture while indigenous sheep were purchased and slaughtered to monitor the prevalent worm species in the region. Results obtained showed that sheep and goats had higher FEC than cattle ($P = 0.000$). The patterns of nematode and trematode FEC in all livestock species did not greatly vary during the study period. There were no significant differences between the sheep and goat FEC ($P < 0.138$). While there were variations in FEC from month to month, the locality of the farms did not appear to significantly affect their counts ($P > 0.05$). *Fasciola* and *Paramphistoma* infestation was recorded in all livestock species. The following genera were recovered from faecal cultures: *Haemonchus*, *Trichostrongylus*, *Oesophagostomum*, *Strongyloides*, *Cooperia*, *Bunostomum* and *Nematodirus*. The two commonest genera were *Trichostrongylus* and *Haemonchus* with *Trichostrongylus* accounting for 40-60 % of the larval recoveries in all livestock species. Data obtained from this study suggested that parasite eggs deposited on pasture in this region at any time of the year had a potential to develop into infective larval stages which had the potential of infecting livestock indicating that the prospects for a strategic anthelmintic control programme in the region was poor. Other means of control such as use of medicated feed blocks are suggested as alternative to strategic anthelmintic control.

Persistent efficacy of doramectin pour-on against experimental nematode infections in cattle

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A programme of eight studies was carried out to determine the period of prevention of infection afforded by treatment with doramectin pour-on at a dosage of 500 µg/kg against artificial challenge infections of *Cooperia oncophora*, *Ostertagia ostertagi* and *Dictyocaulus viviparus*.

For each study, between 31 and 50 worm-free cattle were allocated at random to a control group and one of three treated groups. The animals within each treated group received the prescribed dosage of doramectin pour-on, the timing of the treatments for each group being staggered such that three persistence intervals could be evaluated within each study. The duration of the trickle challenge administered to all animals within a study was the same, and varied between 14 and 21 days, depending on the study. The persistence intervals evaluated ranged from 14 to 56 days. For each study, the percentage reduction in worm burdens for each doramectin-treated group, compared with that of the non-medicated control group, was estimated by using geometric mean worm burdens. An analysis of variance was performed for each nematode species across all studies to establish a single overall figure for the percentage reduction in worm burdens at each persistence interval. The percentage reductions in *C. oncophora* burdens were 99.7%, 95.8% and 88.6% for challenge infections administered up to 21, 28 and 35 days respectively following treatment. For *O. ostertagi*, the corresponding figures were 100%, 99.8% and 98.9% for the same intervals. In the case of *D. viviparus*, burdens were reduced by at least 98.3% for challenges continuing up to 42 days following treatment. It is clear from this data that doramectin pour-on administered to cattle at a dosage of 500 µg/kg of body weight protects against infections with *C. oncophora*, *O. ostertagi* and *D. viviparus* for at least 28, 35 and 42 days respectively.

Isotype-specific antibody responses to *Cooperia punctata* in infected and challenged calves

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The aim of this study was to examine the dynamics of the infection and the serum IgG, IgG1, and IgA responses of calves infected and challenged with *Cooperia punctata*. Twelve 5 month-old Holstein male calves were divided in two groups A and B. Group A was infected with a single oral dose of 130000 L3 of *C. punctata* and Group B was kept as non-infected control. On Day After Infection (DAI) 100 the calves received an anti-helminthic treatment. At a second phase, from the same animals then 11 months old, 4 calves from Group A were re-infected with a single dose of 260000 L3 of *C. punctata*. Three calves from Group B (B.1) were prime infected with the same dose (260000 L3) and a third group (B.2) with 2 animals was non infected control. The infection was monitored by daily faecal egg counts (epg) and weekly blood collection. The pre-patent period was 14 days on both phases. The calves prime-infected at the age of 5 months showed a mean of 3000 epg at DAI 24. Two animals from Group A which showed the higher epg counts died with a correspondent worm burden of 16000 and 28960 adults of *C. punctata*. Re-infected calves, at the age of 11 months, had no eggs on faeces during the 6 weeks of observation. From the calves prime infected on the second phase, one reached 1750 epg on DAI 20, the other maintained a low epg and the third animal had no eggs in faeces. The non-infected control group did not excrete eggs at any time. The dynamics of the humoral response (IgG, IgG1, IgA) against *C. punctata* adult antigen was measured by ELISA (10mg/ml). IgG and IgG1 values showed a similar pattern during the whole experiment. At the first phase, the response to IgG and IgG1 elevated gradually until DAI 56 being stable until DAI 100 when they were treated. IgA levels reached a peak on DAI 21 keeping high values until DAI 56 when a small and gradual decrease until DAI 100 occurred. At the second phase, on DAI 0 the re-infected animals showed high levels of IgG and IgG1 that increased promptly on DAI 7 until DAI 42 while the prime-infected calves exhibited higher levels since DAI 14. IgA values were low on DAI 0 at the re-infected animals and elevated suddenly on DAI 7 keeping high levels until the end of the experiment. Differently the response of the prime-infected calves reached a peak only on DAI 21. Protective immunity was recognized in the challenged animals by no faecal egg counts and pronounced levels of total IgG, IgG1 and IgA.

Schistosoma japonicum in pigs: a model for human and livestock infections

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Control programmes for the zoonotic trematode *Schistosoma japonicum* have been unsuccessful in certain areas of Southeast Asia, notably where livestock hosts play an important role as reservoirs of the parasite. Novel approaches must be found in order to control *S. japonicum* in these areas as the conventional methods of chemotherapy and intermediate host elimination have not been sufficiently effective. Attention is now focusing on natural host-*S. japonicum* interaction studies which can provide information helpful for formulating mathematical models and thus make it possible to identify periods in the parasite's lifecycle when it may be vulnerable to control efforts. Vaccine development is also being given a high priority resulting in the identification of several prospective vaccine candidates which now await testing in animals.

Recent investigations concerned with the population biology of *S. japonicum* indicate that the pig can be used successfully as a host model for schistosomiasis japonica. In addition, their usefulness for testing *S. japonicum* vaccine candidates is receiving increasing attention. The pig may serve as an appropriate and relevant model for schistosomiasis japonica in both livestock and humans since it is a natural host of the parasite and is biologically very similar to humans. Pigs are highly prolific due to their short reproductive cycle and large litter size and can be housed and maintained cost-effectively, thus making them an ideal large animal for experimental studies compared to other livestock.

A geographic information system forecast model for strategic control of fasciolosis in Ethiopia

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A GIS forecast model based on moisture and thermal regime, soils and terrain features was developed to assess risk of *Fasciola hepatica*, a temperate species, and its tropical counterpart, *F. gigantica*, in Ethiopia. Agroecological map zones and corresponding environmental features that control the distribution and abundance of the disease and its snail hosts were exported from the FAO Crop Production System Zones (CPSZ) database on East Africa and used to construct a GIS using ATLAS 3.0 software. The model was validated by comparison of risk indices and environmental features to available survey data on fasciolosis. A remarkable spatial and mathematical correlation was obtained between known infection prevalence and forecast indices. Statistically significant relationships ($P < 0.05$) were found between recorded prevalence values and environmental features [annual rainfall, readily available soil moisture, terrain slope, water logging-ponding hazard and mean Normalized Difference Vegetation Index (NDVI)]. The effect of surplus water was significant ($P < 0.05$) at altitudes above 1900m. Potential Evapotranspiration (PET) was inversely related to prevalence ($P < 0.001$) at below 1950m elevation. *Fasciola* risk indices for 4 climatic regions were used to project transmission patterns under varying climatic conditions. Base temperatures of 10°C and 16°C were used for *F. hepatica* and *F. gigantica*, respectively, based on the growing degree day concept and moisture availability. Distinct regional *F. hepatica* transmission patterns were found. Transmission occurs in the humid west from May-October and in the south from April-May and/or September-October, depending the annual abundance of rain. In the Northcentral region, risk is highest during heavy summer rains and transmission occurs during August-September, except in a wet years, when it may start as early as July and extend through October. The central highlands follows a similar trend, except at cooler sites > 2800m, where completion of an infection cycle may require more than a year. *F. gigantica* risk was present in all agroclimatic zones, except the central highlands, at altitudes of 1440 to 2560m. A transmission cycle can be completed in a single year only at < 1700m. Regional strategic

Is faecal nematode egg count predictive for productivity in traditional West African cattle herds?

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A large scale longitudinal study was undertaken to investigate the effects of a strategic treatment of gastrointestinal nematodes on productivity of N'Dama cattle under village conditions in The Gambia. Initially 1046 animals from 26 private N'Dama cattle herds, were monitored from October 1989 to December 1994. Each herd was stratified by age and the animals were randomly allocated to two groups. The herds were subdivided into two different treatment schemes. In the herds of scheme 1, the treated animals (Treatment group I) were treated once (PanacurTM 7.5 mg/kg BW, Hoechst Veterinär GmbH) in August, whereas in the herds of scheme 2, the treated animals (Treatment group II) were treated twice (in July and September). The same treatment schedule was used in the subsequent rainy seasons until December 1994. One group in every herd of both schemes served as control (Treatment group 0) and received no anthelmintic treatment throughout the study. Annual calving rates of twice treated animals were 52.2 % compared to 43.6 % in the control group ($p < 0.001$, logistic regression), which is an increase of 8.6 % to the control rate. Two annual treatments significantly increase liveweights of the age group 12-24/24-36 months by 9.4 % and 17.5 % respectively. Average weights of three and four year old, twice treated animals are 13.1 % and 8.2 % higher compared to their controls. These productivity improvements are related to a 40 % decrease of mean monthly faecal egg count (FEC) in Treatment group II compared to Treatment group 0. From these results the question arose whether these overall productivity-FEC relationship could be used on a herd or even an individual basis to predict productivity from FEC. Covariance analysis of annual herd calving rates in untreated animals showed no relationship to annual peak FEC. Liveweights of untreated animals at one, two and three years showed no dependence on FEC. Although productivity improvements from deworming are considerable in this study, variation of FEC and the various productivity parameters are too high to reveal a significant relationship.

Continuous *in vitro* cultivation of erythrocytic stages of *Babesia equi* in serum-free medium

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The hemoprotozoan parasite, *Babesia equi*, one of the causative agents of equine piroplasmiasis, was continuously cultivated in microaerophilous stationary-phase culture in a humidified 5 % CO₂-in-air atmosphere at 37°C. The medium used was a modified HL-1 medium supplemented with L-glutamine, antibiotics, and hypoxanthine. Bovine serum albumin (BSA) alone or with chemically defined lipids (CDL) were added in place of serum. Media containing BSA alone or BSA and CDL in various amounts supported the *in vitro* propagation of *B. equi*. Growth was maintained for more than 200 days. Parasites propagated in medium containing BSA could be cryopreserved and resuscitated in the same medium. Once a culture was established almost all stocks tested were easily adapted to serum-free culture conditions. The growth rates obtained with serum-free media were consistently lower than those of control cultures with serum-containing medium.

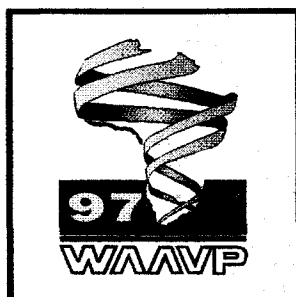
Prevalence and control of sarcoptic mange and helminth infections in pigs in The Czech Republic.

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Sarcoptic mange caused by *Sarcoptes suis* is recognized to be very prevalent disease in swine throughout the world. In our study involving more than 5000 slaughtered pigs in The Czech Republic in 1996 we classified skin of pigs by scoring system for dermatitis associated with mange ADS /average dermatitis score-POINTON et al., 1992/. The prevalence of sarcoptic mange was 54.2% of all slaughtered pigs. Degree 1 /mild dermatitis 46.22%/; degree 2 /moderate dermatitis 7.5%/ and degree 3 /severe dermatitis 0.44%/ and ADS values = 0.62. *Sarcoptes* infestation was confirmed histologically and by positive skin scrapings from pigs.

The health state of pigs of all age groups was monitored simultaneously and faeces samples were treated coprologically. Total 687 faeces samples were examined. The prevalence of helminthiasis was proven in *Ascaris suum* (2.9% - 60.0%), *Trichuris suis* (1.3% - 30.0%), *Oesophagostomum dentatum* (3.3% - 6.8%), *Strongyloides ransomi* (2.6% - 14.3%). Antiparasitics based on doramectin (Dectomax[®]) was administered to pigs therapeutically. The objective of this report was to demonstrate the efficacy of a single injection of doramectin (300 µg.kg⁻¹) in swine naturally infected with *Ascaris suum*. Efficacy of doramectin treatment was assessed between day 11 and day 16 based on the faecal egg count reduction of *Ascaris suum*. The EPG value of *A.suum* decreased from 965 to 0 (day 0 - day 16) respectively in group of swine and treatment against *A.suum* was 100% effective. The high effectivity of doramectin in the control of endoparasites and ectoparasites was demonstrated in pigs under natural conditions in pig farms of The Czech Republic.



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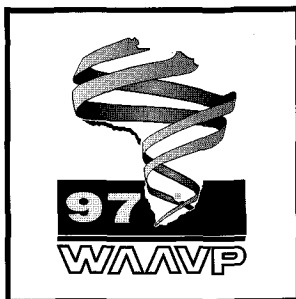
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BREEDING SHEEP WHICH REQUIRE MINIMAL ANTHELMINTIC TREATMENT: A REVIEW OF THE GENETICS OF RESISTANCE AND RESILIENCE OF SHEEP TO NEMATODE PARASITES

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ABSTRACT

MORRIS, C.A. & BISSET, S.A. 1997. Breeding sheep which require minimal anthelmintic treatment: a review of the genetics of resistance and resilience of sheep to nematode parasites.

Two potentially different types of host response are relevant to breeding sheep which require minimal anthelmintic treatment, in the face of nematode challenge. These are "resistance" (ability to suppress the establishment and/or subsequent development of a parasite infection), and "resilience" (ability to maintain relatively undepressed production while subjected to parasite challenge). Most studies have concentrated on resistance, and used faecal worm egg count (FEC) in lambs as the selection criterion. However, in Romney sheep in New Zealand, it has become apparent that "host resistance to parasite infection" does not equate to "host resistance to parasite-induced disease". Genetically low FEC Romney lambs have significantly reduced burdens of the most important nematode species, but they appear to have few significant production advantages over their higher FEC counterparts when all are grazed together under the same larval challenge. Furthermore, there is evidence of an unfavourable association between FEC and dagginess (soiling of the breech area) suggesting that some forms of host response to nematode challenge may result in diarrhoea. The main benefits of selectively breeding Romneys for low FEC are likely to be derived indirectly as a result of reduced pasture contamination by both ewes and lambs. This has led us to investigate the genetics of resilience. While an earlier Australian study had assessed resilience in terms of productivity while infected, relative to productivity while worm-free, this approach was difficult to implement in the field. We therefore chose to use a different approach which involved

assessing the number and timing of drench treatments required to maintain acceptable productivity in lambs, using an individual "on demand" treatment procedure. A total of 213 different rams were progeny tested for this trait as well as for lamb growth rate, dag score, FEC and fleece weight. Overall, the heritabilities of various measures of drench requirements examined were relatively low (ranging from 0.10 to 0.19), although estimates varied between flocks and years possibly as a result of differences in the severity of challenge. There was no indication of an association between the number or timing of drench treatments required by lambs and their resistance to infection. However, results suggested that progeny-test selection for resilience assessed in this way would lead to lambs with higher growth rates and lower dag scores when left undrenched on infective pasture, and this is now being tested in an experimental selection flock. In practice we do not see resilience as an alternative commercial breeding option to resistance, but rather as an additional trait which would need to be combined with resistance if the potential benefits of both traits are to be captured.

Keywords: Dags, diarrhoea, disease resistance, faecal egg count, genetics, growth, nematode parasite, resilience, selective drench treatment, sheep

INTRODUCTION

Breeding sheep which require minimal anthelmintic treatment is one of several options for trying to manage anthelmintic resistance. This may also assist in meeting consumer demands to minimise drug usage in the livestock industries. Although significant progress has been made in understanding the responses of sheep to nematode parasitism, the most

appropriate breeding strategies to achieve the above goal remain unclear. Most early authors recognised that farmers drench primarily to prevent or alleviate the effects of roundworm infection on the health and productivity of their animals, and not simply to eliminate the parasites *per se*. Early reviews and approaches were summarised by Bisset & Morris (1996), highlighting the contrast between "resistance to nematode infection" and "ability to cope with the effects of infection" (resilience). In the last 20 years, most authors studying the genetics of sheep responses to nematode parasitism have concentrated on selecting for resistance to infection, as measured by faecal egg count (FEC), rather than on productivity in the face of challenge, which as pointed out by Albers, Burgess, Adams, Barker, Le Jambre & Piper (1984) may be a more economically meaningful parameter on which to base a breeding programme and assess its benefits. This review discusses some of the practical aspects and implications of the two approaches.

RESISTANCE

There have been numerous recent reviews of the genetics of resistance in sheep to nematode parasites. These have described the genetic parameters, the direct responses achieved in single-trait FEC selection flocks, and the correlated responses recorded in reproductive, productive, immunological or other parasitological traits. One such review (Morris, Watson, Bisset, Vlassoff & Douch 1995) summarised a number of reports of heritability estimates in New Zealand for transformed FEC in lambs, from which a weighted average of 0.23 (s.e. 0.02) was calculated. These estimates were mostly from studies using mixed-species natural challenge on pasture. Somewhat higher estimates have been found in Australia where most studies have been undertaken using artificial monospecific challenge. For example, the mean of 5 estimates of heritability for transformed FEC at intervals (3 to 11 weeks) after infection, was 0.40 (s.e. 0.04) (Woolaston & Eady, 1995). Despite the apparent differences in heritability, the natural or artificial infection protocols in both countries appear to have been used equally effectively in generating nematode-resistant and -susceptible sheep breeding lines. Repeatabilities, or rankings of animals from repeated FEC samples, are relatively high (0.3 to 0.5) for transformed FEC, with the actual value depending on whether the FECs were separated

by a drench treatment, and on the interval between samplings.

Direct responses to selection for high or low FEC in lambs have been documented. Two of the experiments which have continued for longest are the Romney selection flocks at Wallaceville, New Zealand, using natural challenge and beginning in 1979 (Morris, Bisset, Vlassoff, Baker, Watson & Wheeler 1997a), and the Merino selection flocks in Armidale, Australia, using artificial challenge with *Haemonchus contortus* and beginning in 1978 (Woolaston & Piper 1996). Both experiments involve divergent selection flocks, and faster progress has been observed in each for low than high FEC. In the Wallaceville lines, the low-line lambs now produce only ten per cent of the FEC of high-line lambs. These and other sets of selection lines are currently providing a resource for monitoring correlated responses to selection.

FECs in high and low line breeding ewes also differ significantly during the post-parturient period in cases where the high and low lines are grazed together. Breeding ewe FEC also has a medium heritability under New Zealand conditions, estimated at 0.27 (s.e. 0.06) in Perendales (Watson, Hosking, Morris & Hurford 1995) and 0.38 (s.e. 0.06) in Romneys (Morris, C.A. & Bisset, S.A., 1997, unpublished). In the Wallaceville experiment, 68 per cent of the log₁₀FEC divergence of lambs was also recorded in breeding ewes. The lamb and ewe FEC results imply that pasture larval challenge to each selection line will be different when lines are run separately. Research on the epidemiological consequences of breeding for resistance is discussed below.

Post-mortem gastrointestinal worm counts in lambs derived from FEC selection lines also differ significantly for virtually all the economically important nematode species (Bisset, Vlassoff, Douch, Jonas, West & Green 1996a), confirming that selection for low FEC does actually lead to reduced worm burdens. In addition, female worm fecundity differences were found between selection lines, indicating that fecundity as well as establishment is controlled to some extent by host resistance.

Net reproductive rates in selection line ewes have been compared, and there was a significant advantage at Wallaceville of 0.09 (s.e. 0.036) lambs weaned per ewe mated (or 9.8 per cent)

in favour of Resistant over Susceptible ewes (Morris *et al.* 1997a), mainly resulting from advantages in pregnancy rate (although it is unknown at this stage if this is a ram or ewe effect). For correlated responses in body weight, no selection-line differences or paternal half-sib genetic correlations have been found in lambs or in yearlings in Wallaceville or Australian studies (Morris, Vlassoff, Bisset, Baker, West & Hurford 1997a; Eady, Woolaston & Mortimer 1994) or in breeding ewes (Morris, C.A. & Bisset, S.A., 1997, unpublished). However, the results for fleece weight appear to differ among experiments and/or breeds. In New Zealand, Morris *et al.* (1997b) have observed from both paternal half-sib data and realised responses that yearling and ewe fleece weights are positively (i.e. unfavourably) correlated with FEC in Romneys, and the same is observed in Perendales (via realised responses) but not in Coopworths (via paternal half-sib analyses). On the other hand, Eady *et al.* (1994) found no genetic correlation between FEC and fleece weight in Merinos in Australia. The reasons for the apparent breed differences are not yet known.

Unfavourable realised responses have also been observed in dag score in selection-line Romneys (Morris *et al.* 1997a) and in selection-line Perendales (D.M. Leathwick, 1997, personal communication). In Merino ewes under challenge in Victoria, Australia, Larsen, Anderson, Vizard, Anderson & Hoste (1994) could find no significant phenotypic relationship between dags and FEC. However, they did find a strong association between dag score and high eosinophil counts in gastrointestinal mucosa; similarly, higher counts have been recorded in our Romney low FEC line than in the high FEC line (Bisset *et al.* 1996a). Other immunological responses have also been studied in the selection lines, and these were reviewed by Douch, Green, Morris, McEwan & Windon (1996).

From the results of correlated changes described above, there seems little apart from a minor effect on reproduction in ewes favouring the net productivity of Resistant over unselected or Susceptible flocks when all are grazed together on infective pastures (or artificially challenged at the same dose rate). In reality however, the situation is more complex than this. When the genotypes are grazed separately, parasitic challenge will be lower in Resistant-line animals

than Susceptible-line animals, because of the lower pasture contamination rates associated with low-FEC ewes and lambs (Bisset, Vlassoff, West & Morrison 1997). Modelling studies are underway in New Zealand and Australia to attempt to predict the long-term population dynamics of the nematode to different management and host selection practices. In addition, individual farmlet studies of lambs have recently been undertaken, which have demonstrated some advantages to resistant selection-line lambs when grazing separately (Bisset *et al.* 1997; Woolaston, Barger & Eady 1997). A replicated farmlet study involving both ewes and lambs, where selection lines have been separated year-round, has been established with Perendale selection-line animals in New Zealand since September 1995 (D.M. Leathwick, personal communication). Early results of this study are awaited with interest.

RESILIENCE

The fact that genetic factors are involved in the ability of sheep to cope with the effects of nematode challenge has been known for many years. Whitlock (1958) was among the first to demonstrate genetic variation amongst sheep in this trait. He showed significant differences among individual sire progeny groups in haematocrit levels following natural challenge with *H. contortus*, although no heritabilities were calculated. More recent results (Piper 1987) have provided a heritability estimate of 0.25 (s.e. 0.13) for packed-cell-volume-decline in infected adult Merinos.

However, the first real attempt to study resilience in sheep and its relationship to resistance was undertaken by Albers *et al.* (1984) and Albers, Gray, Piper, Barker, Le Jambre & Barger (1987) in Australia and this related to *H. contortus* infections in Merino sheep. Their results indicated that the heritability of resilience (which they measured in terms of depression of growth rate while infected) was substantially lower than that of resistance (0.09, s.e. 0.07; c.f. 0.26 to 0.34, s.e. 0.10) but also that the two traits were favourably associated, with a moderately strong genetic correlation of 0.56 (s.e. 0.23). It thus seemed to those authors that selection for either trait would ultimately achieve a similar end point. In view of the fact that their results also indicated that the heritability of FEC was substantially higher than that of resilience, it appeared that breeding for resistance to infection would be the

more rewarding strategy to follow. This view was further supported by Piper & Barger (1988) who pointed out that, as resistance to *H. contortus* in Merinos was genetically independent of production in a parasite-free environment, selection for increased resistance in a "parasitised" environment should lead to an increase in productivity on the basis that "sire groups that suffer the greatest worm burdens also tend to suffer the greatest production losses".

Unfortunately however, as seen from the New Zealand results described above, the scenario predicted by Piper & Barger (1988) for Merinos is less straight-forward in Romneys and some other dual-purpose sheep breeds. For this reason, in 1991 we initiated genetic studies of resilience to complement our studies of host resistance. Because the method used by Albers *et al.* (1987) to assess resilience was difficult to implement in the field, we considered a range of other ways to examine the trait (see Bisset & Morris 1996). As a result we chose to record resilience using a novel approach which involved assessing the number and timing of drench treatments required to maintain acceptable health and productivity in lambs under field conditions. This was accomplished using an individual "on demand" (i.e. selective) drenching procedure based initially on visual assessment of lamb condition (Bisset, Morris, Squire, Hickey & Wheeler 1994) and subsequently on weight gain criteria (Bisset, Morris, Squire & Hickey 1996b). While essentially reflecting productivity under nematode challenge, this approach avoided the ethical problem of leaving less resilient animals untreated during an extended test period, and also recognised that our ultimate breeding objective was to reduce reliance on drenching to control parasitic disease.

One disadvantage of the selective drenching approach is that it can lead to complications in ranking animals for other traits such as productivity and/or resistance to infection, both of which require that all animals are treated alike. For this reason and because one of our aims was to investigate the relationship between drench requirements and host resistance in lambs, only male progeny groups in our studies were evaluated for resilience using the selective drenching procedure. This allowed resistance and standard productivity traits to be measured at the same time in their paternal half-sisters under the more traditional "trigger drenching" (i.e.

drench all animals when a threshold FEC level has been reached by a monitor group). The ram-lamb progeny groups were used to provide progeny-test results on their sires for (i) the age at first drench, (ii) the proportion drenched at least once by a given time, and (iii) the average number of drenches by a given time. These three factors were found to be closely correlated with each other genetically (average = 0.87), so they could be taken as essentially measuring similar or closely correlated traits.

Estimated heritabilities of the resilience or drench requirement traits examined in our exploratory work over the first three years are shown in Table 1. Values are based on data from male lambs from a total of 11 flock-year combinations. For comparison heritability estimates in females are shown for weight gain, autumn live weight and dag score, along with separate heritability estimates for the resilience traits in males from one particular flock (Flock 2). Overall our heritability estimates for resilience, like those of Albers *et al.* (1987), were relatively low compared with those for standard productivity traits, although in our case standard errors were small and all estimates were significantly different from zero. By comparison, estimates from Flock 2 were considerably higher than the overall values. This is believed to reflect the fact that male lambs in Flock 2, in contrast to those in the other flocks, received no anthelmintic treatment at weaning, and throughout the test period were consistently subjected to higher levels of cumulative challenge prior to receiving drench treatment. That is to say higher challenge levels in this flock possibly resulted in clearer discrimination among progeny groups, revealing greater genetic variation.

In calculating the above heritabilities, it was necessary to adjust the resilience traits for known non-genetic effects. Bisset *et al.* (1994) found that such factors included lambs' birth date (with earlier lambs tending to cope better under challenge than those born later); birth rank (with single born lambs tending to cope better than those born as twins or triplets); and age of dam (with those born to middle aged ewes tending to cope better than those born to young ewes (2 years old) or aged ewes (>4 years old)). In general these factors had their effect via their influence on weaning weight.

TABLE 1 Heritability (h^2) estimates for select-ed resilience and productivity traits in male Romney lambs: 1991–93 crops

Trait	All flocks ¹		Farm 2 only	
	h^2	s.e.	h^2	s.e.
Number of sires	(213)		(64)	
Age at first drench	0.14	0.03	0.34	0.07
Proportion drenched by time 2	0.10	0.03	0.24	0.07
Total drenches given ²	0.19	0.04	0.53	0.13
Weight gain (weaning to autumn)	0.21	0.02	-	
Autumn live weight	0.27	0.03	-	
Dag score	0.23	0.03	-	

¹ 5 flocks born in 1991 recorded; 4 in 1992; 2 in 1993.

² 5 flocks born in 1991 recorded; 1 in 1993.

In a separate study, a total of 89 Romney rams were progeny-tested in the Wallaceville progeny-test flock from 1988 to 1993. These comprised 12 Resilient, 39 Resistant and 38 Susceptible rams (all allocated to one of the three classes before progeny testing). From the records of 2409 progeny (Bisset & Morris 1996), the clear results were that dag score was lowest (i.e. least daggy sheep) in the Resilient group, postweaning gains were 0.7 kg (9 per cent) higher in the Resilient group, and autumn weights were 1.1 kg (4 per cent) higher in the Resilient group than in the Resistant or Susceptible groups. In contrast, FEC in the Resilient group was intermediate between the Resistant and Susceptible groups.

As our results suggested that progeny test selection of rams for resilience should be feasible, we established a resilience selection flock in 1994. Elite rams were chosen using the solution file of breeding values from the original heritability estimates. First results were reported by Morris & Bisset (1996). The combined 1994 and 1995 lamb-crop results have shown an advantage to the Resilient line of 1.9 kg (19 per cent) in post-weaning gain and of 3.0 kg (10 per cent) in autumn weight, relative to Controls. No significant change in dag score has occurred yet. Results of the 1996 lamb crop are currently being assessed; but after three months of selective drenching in

males, 34 percent of the Resilient line were still undrenched, compared with 11 % of the Controls. In 1997, ewe numbers per flock have been increased at mating from about 100 to over 300, in order to intensify the resilience studies.

GENETIC CORRELATIONS BETWEEN RESILIENCE AND RESISTANCE

A number of previous studies have examined the genetic relationships in lambs between productivity under nematode challenge and resistance to nematode infection (i.e. FEC). However, this past information has not given any real indication of the reduction in drenching frequency which might be expected as a result of selection for these traits. In contrast, the selective drenching approach employed by Bisset *et al.* (1994) and Bisset *et al.* (1996b) led to an examination of the relationship between the above traits and drench requirements of lambs under challenge. This in turn provided the opportunity to assess the likely consequences of selection for these traits in terms of expected reductions in drench requirements.

In order to assess genetic correlations between resilience (measured in terms of drench requirements) and standard productivity or parasitological traits, genetic correlations were calculated across the sexes. In doing this it was necessary to assume that genetic relationships between FEC, dag score and production traits were similar for male and female lambs. Evidence to justify this was discussed by Bisset *et al.* (1994).

Genetic correlations between resilience traits and productivity/parasitological traits in two different lamb cohorts (1991- and 1992-born) are summarised in Table 2. Correlations were relatively consistent over both years and indicated that rams whose male progeny required fewer drench treatments to maintain acceptable condition while grazing under natural challenge (i.e. were more resilient) tended to have female progeny which maintained higher than average growth rates and lower than average dag-scores under challenge. As pointed out by Bisset *et al.* (1994), these characteristics are likely to be important if farmers are to accept reduced drench input in lambs. Significantly, correlations between drench requirements and dag-score were just as high in the second year of the study when dags were not specifically taken into account in selective drenching

decisions. This reflected the fact that there tends to be a negative genetic association between dag score and liveweight gain (Bisset *et al.* 1994, 1996b). Correlations in Table 2 between resilience traits and hogget fleece weight also tended to be favourable, whereas those between resistance and hogget fleece weight in Romneys have been consistently unfavourable.

TABLE 2 Genetic correlations between resilience traits in male lambs and parasitological and productivity traits in their paternal half-sisters (born in 1991; 1992 in brackets)¹

Female trait	Male trait		
	Age at first drench	Drenched by time 2	Total drenches given ²
Autumn live weight	0.54 (0.60)	-0.38 (-0.78)	-0.52
Liveweight gain	0.61 (0.55)	-0.58 (-0.58)	-0.54
Hogget fleece weight	0.21 (0.17)	-0.27 (-0.51)	-0.21
Dag score ³	-0.68 (-0.69)	0.60 (0.73)	0.53
Log _e (FEC + 100)	0.16 (0.01)	-0.16 (-0.14)	-0.17

1 1991: 123 sires; 1992: 100 sires: data relating to 1991 lambs after Bisset *et al.* (1994).

2 Only 1991 data.

3 Greater scores indicate more dags.

Genetic correlations between resilience to nematode challenge in the male lambs and resistance to nematode infection (FEC) in their paternal half-sisters were not significantly different from zero, with the estimate being -0.17 (s.e. 0.18). Thus, in contrast to the results of Albers *et al.* (1987) for sheep-*H. contortus* interactions, our results imply that selection for increased resilience in Romney lambs under mixed species larval challenge would produce little or no genetic change in FEC. On the same basis selection for reduced FEC under the same conditions would be expected to produce little or no genetic change in drench requirements under challenge.

CONCLUSIONS

1. Resistance to infection (FEC) is heritable. Selection against FEC in lambs would lead

to lower FEC in both lambs and breeding ewes, but it also may result in increased dags. At least in Romneys and Perendales there is likely to be a small associated cost in reduced fleece weight in yearlings and ewes. Grazing resistant animals by themselves may lead to some increase in productivity, but the potential saving in drench requirements for this strategy is as yet uncertain. Studies currently underway should answer this question.

2. Due to low heritability levels, selection progress for increased resilience using our criteria would be slow, but nevertheless feasible if progeny testing is employed. As the genetic correlation between our resilience measures and FEC is not significantly different from zero, selective breeding for resilience would have little or no effect on worm egg output and thus pasture contamination rates, at least in Romneys under conditions where the predominant species of nematodes are *Trichostrongylus* and *Ostertagia*.
3. In order to improve both productivity under nematode challenge and reduce FEC through selective breeding, either sequential selection or an index incorporating both traits would need to be applied.

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RAMPANT ANTHELMINTIC RESISTANCE IN SHEEP IN SOUTH AFRICA — WHAT ARE THE OPTIONS?

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ABSTRACT

The latest surveys of anthelmintic resistance suggest that South Africa may be one of the worst affected countries in the world. Despite this fact, and indications that anthelmintic resistance may progressively make sheep farming uneconomical, South Africa is seriously scaling down research into gastrointestinal nematode infections. Given the constraints on funding, two main approaches are suggested by us for managing resistance in South Africa:

Firstly, to use clinical appraisal of the colour of the ocular mucous membranes of sheep (termed the FAMACHA[®] method) for identifying animals not able to cope with *Haemonchus contortus* infection. Preliminary trials indicated a significant correlation of FAMACHA[®] categories with corresponding packed cell volumes of sheep, and that this FAMACHA[®] system is cheap to apply and easily taught, even to illiterate persons. The system presents a practical way to identify the most resistant/resilient sheep, as well as those individuals unable to cope with *H. contortus* infection. It also makes it possible to drench only those suffering from severe parasitism and leave the others not showing clinical anaemia undrenched, thus selecting less intensively for anthelmintic resistance, than when all the animals in the flock are drenched. A chart was produced, depicting sheep eyes with varying shades of red and pink conjunctivae, and information pamphlets were drawn up for training purposes.

The second subject identified for research with the view to managing resistance, is to dilute resistant with susceptible worm strains. While a successful attempt at obtaining reversion was already reported in 1990, little has been done to overcome practical problems encountered with field application. However, a new grazing system that was developed by pastoral scientists in South Africa (concentrating all the animals on half the pasture, while leaving the rest ungrazed for a year), seems to offer the ideal solution for applying this method on farms where resistance

becomes unmanageable.

Keywords: Anthelmintic, Anthelmintic resistance, Biological control, Anthelmintic resistance management, South Africa, Sheep, Conjunctiva colour, Haematocrit, *Haemonchus contortus*, Resistance reversion.

INTRODUCTION

Recently, Van Wyk, Malan & Randles (1997) posed the question: "How long before some strains of *H. contortus* become impossible to control using only the available anthelmintics?"

The question originated primarily from the first description of a strain of *Haemonchus* resistant to 8 drugs, of which there was at least one representing every one of the 5 modern groups of nematocides. Of the 9 compounds tested, only 1 had apparently undiminished efficacy against this South African strain of *H. contortus*, all the rest being less than 80% efficacious (Van Wyk et al. 1997).

This case gives substance to what Van Wyk suggested in 1983* (published: Van Wyk, 1985) that "... unless we use [the anthelmintics] to better advantage, we may find ourselves in the situation where we no longer have effective remedies available for worm control", and in 1990, that: "... it seems likely that before long there may be worm strains showing resistance to drugs from all 5 [anthelmintic] groups" (Van Wyk, 1990). While at the time this prediction was met with polite disbelief in South Africa, subsequent developments have shown these predictions substantially correct.

RESISTANCE IN SOUTH AFRICA

*At the 75th Anniversary celebration of the Onderstepoort Veterinary Institute in 1983

Even though the first confirmed case of resistance in South Africa was described only in 1975 (Berger, 1975), by the early 1980s it was becoming obvious from observations that resistance was a serious emerging problem, despite the fact that no surveys of resistance had been done. It was more than a decade later that A.Kloeck (Logos - unpublished observations, 1992), Van Wyk & Van der Merwe (1993) and M.O. Stenson (unpublished data, 1995; 1996) undertook faecal worm egg count reduction test (FECRT) surveys (Tables 1 & 2). In the Ermelo survey farms were selected at random, using a grid system of randomisation, while in KwaZulu-Natal the farms tested in Underberg and Mount Curry represented a large proportion of the properties with more than the 200 sheep stipulated as a criterion for the survey.

The data were analysed by the method of Presidente (1985), using the geometric mean percentage reduction in faecal worm egg counts (FECs). This method is more likely to classify drugs as efficacious, than the RESO method (Anonymous, 1989), which estimates not only the efficacy, but also gives an indication of the repeatability of the test. The data shown in Tables 1 and 2 can thus be regarded as more conservative than those of the recent disquieting results of surveys evaluated by the RESO method in Paraguay (Maciel, Giménez, Gaona, Waller & Hansen, 1996), Uruguay (Nari, Salles, Gil, Waller & Hansen, 1996), Brazil (Echevarria, Borba, Pinheiro, Waller & Hansen, 1996) and Argentina (Eddi, Caracostantogolo, Peña, Schapiro, Marangunich, Waller & Hansen, 1996).

The resistance in Paraguay was described as possibly the worst in the world. But Tables 1 and 2 paint a picture for South Africa that is apparently no less serious, especially considering that a more "lenient" method of evaluation was used, and that 40-75% of the strains of *H. contortus* tested in Mpumalanga and KwaZulu-Natal were less than 40% susceptible to at least one of the anthelmintics

TABLE 1 Percentage of worm strains showing varying degrees of resistance to at least one of the drug groups

LOCALITY	Farms (n)	EFFICACY (%)		
		< 95%	< 60%	< 40%
MPUMALANGA ¹		% of strains ³		
Ermelo	26	100	69	46
KWAZULU-NATAL ¹				
Mount Curry	12	100	44	44
Estcourt	5	100	80	40
Underberg	9	100	83	75
VARIOUS ²	28	93	50	46

¹ M.O. Stenson (unpublished, 1995/1996)

² Mpumalanga, Northern Province, Free State & Karoo (Van Wyk & Van der Merwe survey - 1993)

³ Percentage of the strains tested

TABLE 2 Percentage of worm strains showing resistance to 3 (or 4) anthelmintic groups

LOCALITY	Farms (n)	EFFICACY (%)		
		< 95%	< 60%	< 40%
MPUMALANGA ¹		% of strains ³		
Ermelo	26	69 (19)	16 (8)	8 (8)
KWAZULU-NATAL ¹				
Mount Curry	12	75 (8)	8 (0)	0 (0)
Estcourt	5	60 (60)	0 (0)	0 (0)
Underberg	9	78 (0)	0 (0)	0 (0)
VARIOUS ²	28	50 (14)	11 (7)	7 (4)

See Table 1 for legends

tested. Also in the winter rainfall regions, in the south-western Cape, all the strains tested were resistant to drugs from at least one anthelmintic group (R.K. Reinecke, personal communication 1990; J.S. Van der Merwe & J.A. Van Wyk, unpublished observations). Worse still, in the Ermelo District, 8% of the farms had strains of *H. contortus* that were less than 40% susceptible to some compounds from all 4 of the anthelmintic groups on the market in South Africa. Sixteen percent of worm strains were less than 60% susceptible to drugs from 3 of these 4 anthelmintic groups. This should be a very serious warning to the authorities in South Africa to lend serious support to the research and other efforts being made to manage the resistance that

has developed, instead of reducing it to a mere shadow of its former self.

The 8% of farms with worm strains less than 40% susceptible to drugs from all 4 the anthelmintic groups may seem low for presenting much of a threat at present. However, in the worst affected areas movement of sheep between farms is not controlled, and resistant worm strains are widely dispersed. Farmers also tend to sell their sheep when resistance poses a threat to continued economical production (Van Wyk, 1990a). Such sheep are seldom all slaughtered; they are rather sold in batches at auctions, and distributed to numerous farms. Lastly, in so-called veld ram clubs (that compare breeding stock of numerous breeders on communal pasture for selecting the best animals), the best rams are eventually auctioned, often without considering that resistant worms may have been picked up from the communal pastures (Van Wyk, Van Schalkwyk, Bath, Gerber & Alves, 1991).

Economic sheep farming remains heavily dependent on efficacious anthelmintics (Van Wyk, 1990b; Soll, 1997), but it is very unlikely that any new, unrelated anthelmintics will reach the market within the next decade.

MANAGING RESISTANCE IN SOUTH AFRICA

In the light of the rampant resistance threatening economical sheep production in South Africa, every possible avenue of control should be investigated. However, with fast shrinking funds and manpower in the field of animal nematology, principally two approaches are being considered for managing the resistance that is posing a serious threat to sheep production in South Africa:

- * Using a clinical method for identifying animals unable to resist *H. contortus* and its effects;
- * Diluting resistant with susceptible worm strains.

1. THE FAMACHA[®] SYSTEM FOR CLINICALLY IDENTIFYING ANIMALS EITHER MORE SUSCEPTIBLE, OR RESISTANT/RESILIENT TO HAEMONCHOSIS

Sheep vary greatly in their reaction to worm challenge under similar conditions. As put by Bath, Malan & Van Wyk (1996) [translated]: "Which [veterinary] practitioner has not seen large numbers of sheep dying from worm

infection, others are on the point of death, but many in the same flock are apparently in the best of health." The traditional reaction was to immediately drench all the animals in the flock, while it would be more economical and cause less selection for anthelmintic resistance if only the affected animals could be drenched (Besier, 1997). The problem was to find a practical, economical method for identifying these animals unable to cope with the infection. This led one of us (FSM) to reflect on the possibility of using clinical evaluation of the state of anaemia as an indication of infection of sheep with *H. contortus*.

It is common knowledge that the anaemia from overwhelming *H. contortus* infection in sheep presents clinically as "deathly" white ocular mucous membranes. However, it was not realised that the degree of paleness correlates well with corresponding reductions in haematocrit (HCT).

In outbreaks of haemonchosis we observed a wide range of shades of red and pink (from blood red to practically white) in the conjunctivae of sheep. Subsequently, we conducted a trial to see whether clinical appraisal of the colour of the conjunctiva can be used to identify individual animals that require treatment, instead of having to deworm the entire flock when some die, or have high faecal egg counts (Malan & Van Wyk, 1992).

Trial site: The trial was conducted on a farm near Badplaas, in the "Middleveld" of Mpumalanga, in the summer rainfall region of South Africa. It has hot summers, with mild days and cold nights in winter. The irrigated kikuyu grass (*Pennisetum clandestinum*) pastures are ideal for overwhelming infections of *H. contortus* in summer and autumn, unless the animals are drenched every 3 weeks during the peak worm season, from November/December to April/May.

This farm was selected because resistance had developed to drugs from all the anthelmintic groups, with the exception of levamisole, which was practically unaffected despite more than 3 years of intensive use, to the exclusion of other compounds (Van Wyk *et al.*, 1997).

Method: Over a period of 125 days, from March 22 to July 24, routine drenching of 388 dry ewes on the irrigated kikuyu pastures was stopped, and replaced by a system of "treatment as needed". At the start of the trial and twice more thereafter the HCTs of all the sheep were determined. In addition, at weekly intervals the conjunctivae of the animals were inspected and

their colours classified into the following categories: Red, dark pink, light pink, and pale pink/white. Every animal of which the mucous membrane was judged to be any colour other than red, was bled for a HCT determination, and when the HCT was $\leq 15\%$ the animal's life was considered to be in jeopardy unless it was drenched, and it was dewormed with levamisole at a dosage of 7.5 mg kg^{-1} . Records were kept of all the HCT values and the number of times each animal was drenched.

Results: No worm species other than *H. contortus* were recovered from larval cultures and worm isolates from this Badplaas farm in the present, or previous trials (Van Wyk *et al.*, 1997).

None of the sheep died during the trial period, despite the fact that they were exposed to heavy *H. contortus* challenge, and individual sheep were drenched only after the infection had caused their HCTs to fall to low levels. With few exceptions, the HCTs of the animals clinically classified as any category other than red, were less than 20%.

Over the period of 125 days 1% of the sheep had to be treated four times, 2% of them thrice, 7% twice, and 21% once, to "save their lives"; while 69% were able to control the worm challenge without any need for anthelmintic treatment.

Discussion: The method of clinical appraisal was later named by one of us (GFB) for its originator, the *F*Affa *M*Alan *C*HArt (Bath, Malan & Van Wyk, 1997), or "FAMACHA" guide system.

From the fact that a number of sheep had to be treated repeatedly to prevent possible deaths, it can be deduced that the worm challenge was considerable over this trial period. Furthermore, as none of the animals died, it is obvious that the clinical appraisal identified each animal that was unable to cope with the infection.

Subsequent to this trial, further work was done to evaluate the FAMACHA^c method.

Chart: Firstly, a chart was made (Figure), consisting of a series of 5 identical drawings of a sheep's eye, but with each conjunctiva coloured a different shade of red or pink, respectively corresponding to 30, 25, 20, 15 and 10% HCT. Next to each drawing is a band of colour, corresponding to the predominant colour of the mucous membrane on the inside of the lower eyelid. In addition, the chart was supplied with symbols to indicate for each category the

haematological state of the animal and whether or not drenching is advisable. The instructions for using the chart and precautionary warnings are listed in short on its reverse side.

Ease of application: To get an indication of how easy it is to teach others to use the FAMACHA^c, 7 persons (of whom 5 had had no previous experience of the technique) were given a 15-minute demonstration of how the system works, and were then asked, with the help of the colour chart (Figure) to classify each of a group of 30 sheep into the range of haematological categories on the chart. The HCT of each sheep was determined at the same occasion.

While the correlation between the FAMACHA^c classification and the HCT was significant for every person, the predictive value was sometimes as low as 40%. However, when the correlation was redone using values from only those sheep having HCT values of 20% or below, all the correlations were highly significant ($P < 0.001$) and the predictability of the HCT from the clinical evaluation was as high as 77%. Most important, with only a single exception, every one of the sheep with a HCT of less than 20% was classified by every participant into one of the categories where drenching is advised on the chart. In other words, only a single sheep that was possibly at risk and required treatment (as judged by the HCT) was placed by only one of these inexperienced participants (with no "hands-on" experience), in a category for which the chart did not advise drenching.

More comprehensive trials should be carried out to test more animals and evaluators.

THE POTENTIAL VALUE OF THE FAMACHA[®]:

What needs to be considered now, is how the FAMACHA[®] can be applied to best advantage in the field.

As we see it, there are two main approaches that can be pursued with the FAMACHA[®] system: As a means of identifying sheep that need to be drenched, and for breeding animals with resistance/resilience against helminths.

USING THE FAMACHA[®] SYSTEM TO REDUCE SELECTION FOR ANTHELMINTIC RESISTANCE:

The problem with the use of anthelmintics for worm control is that every programme that controls worms effectively, selects for resistance (reviewed by Besier, 1997), and the more efficacious the control, the greater the rate of selection. One possible way to slow the selection, is to leave some of the animals undrenched (Besier, 1997), thus ensuring that some of the susceptible worms escape treatment and continue to produce ova and dilute the resistant genes of the offspring of the worms that survive the drenching.

However, previously there was no practical method for selecting the most suitable animals to be left untreated: FECs are too expensive to apply to every animal in a flock of sheep, and if heavily infected animals are not treated, losses in production, or even deaths may occur. For instance, with *Ostertagia* or *Trichostrongylus* spp. infection, relatively subjective values have to be resorted to, such as "visual assessment of lamb condition and ... weight gain criteria" (Morris & Bisset, 1997). But for *H. contortus* the FAMACHA[®] system can now be used to select animals not coping with the infection. In other words, in contrast to leaving a small proportion of the animals undrenched (as suggested by Besier, 1997), only the few animals being overwhelmed by the infection need to be drenched, leaving the great majority of the flock to propagate the susceptible *H. contortus*. Many worm ova will still be voided on the pasture, but the preliminary trials have shown that the majority of animals can withstand a high level of challenge, and those that do not, should be culled.

Admittedly, it is to be expected that some loss of production may occur when the FAMACHA[®] system is applied as suggested, but the overriding advantage will be **sustainable production** at those levels; it is becoming clear

that sheep production will not be sustainable unless the farmer aims for optimum, instead of maximum production for his circumstances.

It can also be argued that the FAMACHA[®] system may be too labour-intensive if applied as suggested above, as it is regarded by some as impractical to examine sheep every 7-14 days at the height of the worm season. However, in addition to less severe selection for resistance, consider the saving in anthelmintic drenching costs if less than 20% of the flock needs to be treated, and that less frequently than before. For instance, for the trial with 388 sheep near Badplaas (above), Bath *et al.* (1997) estimated that the "drench-by-observation" approach made it possible to reduce the number of drenches from more than 2300 to just over 160 over the period of observation, a reduction of more than 90%.

APPLICATION OF THE FAMACHA[®] FOR SELECTING ANIMALS WHICH CAN WITHSTAND *H. CONTORTUS* INFECTION

Probably the best potential benefit of the FAMACHA[®] is to cull those animals which require "excessive" numbers of anthelmintic drenches, and at the same time to select the ones that are best able to withstand the *H. contortus* infection. Piper (1987) estimated the heritability for HCT reduction in infected Merino sheep to be 0,25.

The low costs of the FAMACHA[®] system make it possible to select not only rams for breeding, but the entire flock. A big advantage is that, as only the animals that are overwhelmed by the infection are drenched, these poorly adapted animals are automatically identified, and not shielded by the usual practice of drenching the entire flock when only some animals need it.

The question is, if the FAMACHA[®] system does prove itself, what role it can play in the present system, in which animals with low FECs and/or high antibody levels are selected for breeding resistant sheep. This remains to be determined, but it is envisaged that for the more valuable animals (such as male breeding stock), both systems will be used together.

The relationship between the HCT and the FEC in animals under stress was investigated in 112 sheep that were very heavily infected with *H. contortus* (F.S. Malan & J.A. van Wyk, unpublished observations). Some in the flock

were dying from natural *H. contortus* infection, and the FECs of individuals among the 112 sheep were as high as 56 000 g⁻¹. At the laboratory the HCT and FEC of each animal was determined.

While the correlation between the HCT and the FEC was significant ($P < 0.05$), the HCTs were but poorly predictable ($r = 0.197$) from the FECs: By linear regression studies it was estimated that only 3.9% of the variation of HCT values of the sheep was predicted by the FEC. Thus it seems likely that the two phenomena are controlled by different mechanisms in the sheep. This is in agreement with our opinion that, possibly with some individual exceptions, the HCT is not only a measure of the sheep's resistance to infection with *H. contortus*, but also of its resilience, in which case such sheep can be identified more easily and cheaply and perhaps more accurately than in the case of the *Ostertagia* and *Trichostrongylus* spp. mentioned.

Unfortunately, if animals need to be stressed until the HCTs of some drop to low levels, the FAMACHA[®] system may not be popular with some farmers. However, firstly, it should not be necessary to stress the whole flock more than once or twice initially, to identify the adult culls and breeding animals; thereafter, only the lambs need be stressed for continually utilising the advantages of the FAMACHA[®] system. Another possibility that seems to be emerging from our trials, is that the rebound in the HCT after drenching with an effective anthelmintic may be of use for selecting resilient animals. When the 112 sheep mentioned above were drenched with compounds with widely differing levels of efficacy, within a week there were increases in HCT, as high as 7% in those sheep treated with a highly effective anthelmintic (F.S. Malan & J.A. van Wyk, unpublished observations). This should be followed up, in case double HCT determinations (with an anthelmintic drench inbetween) may make it possible to select the animals before the HCTs have dropped to low levels.

While admitting that some systems for reducing the selection for resistant worm strains may be unpopular, we are of the opinion that many farmers probably have little choice left if they wish to continue to produce sheep economically in South Africa. Thus, systems that may now seem unlikely to be accepted by farmers, may be of use when there are no other options, other

than to abandon sheep farming.

PLANNED APPLICATION OF THE FAMACHA[®] IN SOUTH AFRICA: Plans are being laid by the W⁶ (South African Worm Resistance Workshop), the Faculty of Veterinary Science of the University of Pretoria, and the Onderstepoort Veterinary Institute to disseminate knowledge of the FAMACHA[®] in South Africa, with the view to wide application in the field, for sustainable sheep production. However, it is necessary firstly to identify veterinary practitioners and, through them, farmers who are willing to take part in a thorough evaluation of the pros and cons of the FAMACHA[®] under practical farming conditions.

After informal farmers' meetings at which the general acceptance of the concept was tested, a set of pamphlets was produced by one of us (GFB), providing information on the FAMACHA[®] system and trials intended for launching in the 1997/8 worm season in South Africa. One information pamphlet is appended to this paper, for the edification of persons possibly interested in applying such a system (see appendix).

Initially it is intended to test the system mainly on selected farms for an entire worm season, thereafter to adapt it as necessary, and then to apply it more widely.

II. REVERSION OF ANTHELMINTIC RESISTANCE

As discussed, workers in this field stress that sheep production is precarious without efficacious anthelmintics (Van Wyk, 1990; Soll 1997). Also, new anthelmintics (Soll, 1997) and alternative methods of control, such as vaccines (Smith, 1997) are being developed so slowly, that sustainable sheep production will be extremely difficult unless the existing anthelmintics can be given a new lease on life. And even if new, unrelated anthelmintic groups were to reach the market, in view of the recent history of new introductions, it seems very unlikely that it would provide anything but temporary relief.

What, then, remains for relieving the threat to economical sheep and goat production on the farms having almost no effective drugs left? To our knowledge, there are only two possible alternatives available: Either to remove susceptible animals from the pastures infected with the resistant worm strains for at least 12–24

months (depending on the worm species involved), or to attempt to dilute resistant with susceptible worm strains.

a) Total withdrawal of susceptible animals from pasture: At first glance, if economically viable (which often it would not be), it would seem a good option to leave pastures unutilised by susceptible animals until all the resistant worm free-living stages have succumbed. However, on closer scrutiny this is not so simple. How are the animals to be selected, which are introduced after the period of rest of the pastures? Resistance is so common in the countries where such desperate measures for managing resistance would be considered, that another resistant worm strain will most likely be introduced with the sheep that are brought in after the period of rest.

It can be argued that the sheep can be thoroughly dewormed before they are introduced, but it is practically impossible to ensure that all worms are removed by the treatment. In any case, it is not a viable option to attempt to farm worm-free, as worm larvae can easily be introduced from elsewhere (for instance, by flood water, birds, faeces, stray animals, man) and, once again, these are likely to have at least some resistance. Keep in mind that *H. contortus* is such a prolific egg layer, that even a few remaining worms will most likely "restock" the farm with worms within a year or two.

Otherwise, it could be considered to deworm the animals as suggested above, and then deliberately to infect them with a susceptible worm strain, so that the rested pastures are seeded with susceptible worms known to have given no indication of resistance. But this corresponds with the dilution of a resistant strain, as discussed below.

b) Diluting a resistant worm strain on pasture: Van Wyk & Van Schalkwyk (1990) managed to get reversion of a relatively resistant strain of *H. contortus* on pasture to a more susceptible one, by intervening in spring, when the pasture is apparently not infective on the Highveld of Gauteng and Mpumalanga Provinces.

This potential method for reversing resistance has not received serious attention internationally and has been criticised (Van Wyk, personal

observations) as being impractical and unacceptable to farmers, but keep in mind that all other methods aimed at delaying the development of anthelmintic resistance are to no avail if no efficacious drugs remain for controlling the worms on a given farm. In other words, once again the farmer may have no choice.

What is required regarding research in a situation of rampant resistance in many countries of the world, is firstly to find something that apparently works, and then to investigate means of making it practical for field application.

In South Africa pastoral scientists have developed a new system of grazing management, that may make it easier in practice to dilute resistant worms strains on pasture. Kirkman & Moore (1995) showed that on the Highveld of Mpumalanga the best method for sustainable improvement in the quality of natural pasture herbage is what they termed the "50/50" system, where all the animals are concentrated on half the available pasture, while the remainder of the property is left ungrazed for at least one full growing season, but usually for a full year. This means that practically worm-free pasture is available almost annually, and this seems to offer an ideal situation for applying this method (Moore & Van Wyk, 1997).

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Appendix

FAMACHA^c INFORMATION PAMPHLET

Compiled by the W⁶ (Worm Workshop of the SAVA)+ Hoechst Roussel Vet + Pretoria University's Onderstepoort Faculty + the Onderstepoort Veterinary Institute

NOTE:

1. It is essential to read and follow all the instructions and understand the information supplied so that the user can obtain maximum advantage from use of the FAMACHA^c Guide.
2. This is at present an experimental concept and the user must undertake to use it at his or her own risk.

WHY THE FAMACHA^c WAS DEVELOPED

- * Wireworm is the biggest disease problem of sheep in most of the summer rainfall areas of South Africa
- * Resistance to worm remedies is an ever-increasing problem, to the extent that on many farms there is resistance to all the groups of drugs and the viability of sheep farming is threatened.
- * While most sheep are able to withstand the unfavourable effects of wireworm, a small minority of sheep cannot. In the past, dosing strategies have been designed around the minority of sheep which do not have resilience to infection
- * Resilience (the ability to withstand the effects of parasites) has been shown to be heritable, although not at a high level.
- * By identifying sheep unable to cope with reigning worm challenge infections, they can be targeted for special attention without having to treat the whole flock. In the long term, by culling sheep which are repeatedly identified as unable to cope, a more resilient flock genetically suited to the environment can be bred.

THE PRINCIPLE ON WHICH IT IS BASED

Blood consists of a clear, fluid part (called plasma) and a cellular part (mainly red blood cells). The proportion of red cells to plasma determines whether the animal is healthy (normal) or unhealthy (too little or too much red cell content). This proportion can be measured in the laboratory by special tests, but with training and practice can also be estimated fairly accurately by assessing colour changes of the ocular mucous membranes. As wireworm is a blood sucker, the effects of a heavy parasite burden in non-resilient sheep will therefore be seen as a low ratio of red cells to plasma. This is seen in mucous membranes as a visible paleness known generally as anaemia. By monitoring anaemia we can identify which sheep are resilient and which are susceptible.

USES AND ADVANTAGES

- * Sheep which are unable to cope with wireworm infection can be identified.
- * These sheep can be treated before the symptoms and effects become too severe, if the flock is examined regularly enough.
- * Identified anaemic sheep can be given the correct drug, if necessary at a higher dose, or in split doses, as there will probably be only a small number of sheep to be treated at each examination.
- * Individual sheep that repeatedly fail to cope with wireworm in spite of an effectively designed control programme can be identified and eliminated from the flock.
- * Sheep which escaped dosing or were under- or improperly drenched (e.g. owing to faulty drenching syringe, etc.), can be identified before severe problems occur.
- * Pasture contamination by wireworm larvae can be reduced.
- * Because fewer sheep are being treated, the development of resistance in worm populations will be slowed down.
- * In the long term, elimination of non-resilient sheep will allow for the breeding of better adapted sheep.

- * A major drop in the amount and frequency of dosing can be expected for the majority of the flock on farms where the helminth burden is high.
- * If an ineffective remedy for wireworm was used, this would be apparent because many anaemic sheep would still be seen. However, if an effective remedy is used, pale mucous membranes should become noticeably redder in colour within a week.
- * If a severe buildup of infective larvae occurred, an early warning of the impending danger could be observed.
- * Paddocks or camps which repeatedly give problems can be identified for taking action.
- * The technique, once learned, is relatively cheap if labour costs (which should be reckoned as fixed costs) are not considered.
- * The technique is very easy and reasonably reliable once learned under the guidance of a competent instructor.
- * long way with no rest period afterwards; any fever; infectious eye disease; diseases associated with blood circulatory failure.
- * The sheep have to be monitored regularly (at least every second week, preferably weekly at the peak of the worm season) to keep a check on developments.
- * Lambs are very susceptible and need special attention.
- * As this technique is still in the experimental stage, no guarantees can be given or are implied. Neither the Worm Workshop, its members nor agents can be held liable for losses which may result from the use of this technique.

PRACTICAL USE OF THE FAMACHA^c

PRECAUTIONS AND DISADVANTAGES

- * Only wireworm infection can be monitored using this technique. A programme for controlling other worms must be used as well.
- * A good integrated wireworm control programme must still be used; the FAMACHA^c will only augment, not replace, this control programme.
- * Flock faecal egg counts must be taken on a regular basis (every 4-6 weeks).
- * There are other causes of anaemia which can cause confusion. Some examples are: Hookworms; liver fluke; external parasites; blood parasites; infections; and nutritional deficiencies. By far the most important cause of anaemia in sheep in South Africa is, however, the wireworm.
- * On the other hand, certain conditions can make the eye's membranes appear redder than expected and thus mask the presence of anaemia. Some examples are: dusty conditions or closed stables, which irritate the eyes; hot conditions; animals driven a
- * Use in conjunction with a properly designed integrated control programme drawn up by a veterinarian.
- * In the first half of summer, institute a proper strategic dosing programme in conjunction with monitoring of faecal egg counts, a rotational grazing system and alternation of grazing with cattle or horses.
- * In the first half of summer (October to December in most sheep-raising areas) carry out the examination of the flock using the FAMACHA^c every 2 weeks by properly trained persons, fully competent to see changes indicating anaemia.
- * In the second half of summer, or even earlier in areas with mild climates and high humidity, rainfall or irrigation, it may be necessary to monitor the flock on a weekly basis.
- * Continue with the integrated worm control programme through to the end of the danger period for wireworm (from the end of March to even June, depending on temperatures and rainfall).
- * Any sheep which become clearly anaemic (D or E categories on the FAMACHA^c chart, and doubtful cases of C) should be dosed with an appropriate remedy (in consultation with the supervising veterinarian) and marked or identified in some permanent

way.

- * If a large proportion (>10%) of the flock are found to be anaemic at a given examination, it may be advisable to dose the whole flock and change camps if appropriate. Consult your veterinarian if in doubt.
- * Sheep identified as needing **more than one** extra dose can be considered for culling, while those needing **more than two** extra doses should definitely be culled.

THE FUTURE OF ANTHELMINTIC THERAPY FROM AN INDUSTRY PERSPECTIVE

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ABSTRACT

Parasitic infections continue to play a significant role in limiting the ability of livestock to realize their full productivity potential and the sustainability of production systems is influenced by our ability to control this threat. Chemotherapy continues to serve as the cornerstone of parasite control, but effective antiparasitic compounds constitute a limited resource and their future availability will be impacted by a variety of factors, including continued escalation of research costs, increased regulatory requirements, development of resistance, and attrition of older products, which fail to meet more stringent modern regulatory and manufacturing requirements. Approval and usage of such antiparasitic products may be further impacted by socio-economic justification for usage, animal welfare issues and human food safety concerns. Limited growth or reduction in animal numbers in many markets and integration and consolidation of production systems can also be expected to result in fewer, more sophisticated consumers. In the absence of new chemical classes, emphasis will be placed on formulation diversification and optimization of product usage in integrated control strategies. There will also be increased interest in exploiting non-chemotherapeutic approaches, but widespread commercial applications of these approaches may be slow to develop. Industry will continue to seek new compounds, but even when these are brought forward, the challenge of the future will be to implement effective and rational parasite control programs in an environment of evolving production systems and socio-economic conditions.

Keywords: Anthelmintic, Anthelmintic development, Anthelmintic resistance, Novel anthelmintics, Anthelmintic sales.

INTRODUCTION

Parasitic infections are a major constraint on livestock production in many parts of the world and, despite implementation of various control

strategies, parasitism is still one of the major health problems confronting the industry. Based on the sequential availability of a series of highly effective antiparasitic compounds over the last 3 decades, producers have come to rely on effective anthelmintic treatment to control the effects of endoparasitism. The threat and reality of anthelmintic resistance has focussed attention on sustainable parasite control, and developing and implementing systems which do not rely on drenches alone to achieve control. Progress continues to be made with non-chemotherapeutic approaches such as vaccination (Emery 1996), selection for resistant hosts (Woolaston, Barger & Piper 1990) and biological control (Grønvold, Henriksen, Larsen, Nansen & Wolstrup 1996) but practical application has been slow to develop. It seems highly likely that these approaches will mainly be effective as a component of integrated control programs, in which effective chemotherapy will continue to play a major, essential role. Even the effective strategic and tactical treatment programs based on epidemiological principles that continue to evolve, are also reliant on effective anthelmintic treatment, thus emphasising the continued importance of chemotherapy as a cornerstone of effective parasite control for the foreseeable future.

There is probably no single sustainable method of parasite control and long-term control will rely on continual replacement of one method by another. Chemotherapy is obviously no exception, particularly as development of anthelmintic resistance constitutes a major threat. It is well-accepted that resistance to benzimidazoles and levamisole/morantel anthelmintics is common (Overend, Phillips, Poulton & Foster 1994) and, although widespread resistance to the avermectin/milbemycin class has been slow to emerge (it was first identified in the field a decade ago — Carmichael, Visser, Schneider & Soll 1987; Van Wyk & Malan 1988), recent developments within the sheep industry of South Africa (Van Wyk 1990) and Latin America have led to a

heightened awareness of the threat of resistance to this class (Waller, Echevarria, Eddi, Maciel, Nari & Hansen 1996; Bridi, Carvalho, Cramer & Eagleson 1997).

There is an expectation that new chemical entities which will provide effective control in the face of evolving resistance patterns will be discovered and developed, but, because this process is invariably difficult and lengthy, there is a need for development of strategies to sustain control measures currently available and to utilize emerging concepts appropriately to maximize their utility (Barger 1993; Donald 1994).

Novel Anthelmintics

Discovering and developing new chemical entities is an arduous, costly and time-consuming process. In order to assess the prospects for successful development of a novel anthelmintic, it is important to consider a number of issues, which confront any development program.

Discovery may be based on compound screening in biological and/or in vitro systems or on targeted drug development through molecular modelling and receptor characterization. To date, the majority of new chemical entities have been discovered through screening systems, although optimization of the molecular process provides encouraging prospects for the future.

Following identification of an active compound, an effort is made to optimize and alter activity by chemical modification, followed by further testing to establish utility. Pre-clinical trials and tests to establish safety for consumers are conducted and it is in this safety testing process that many candidates fail. These studies include a battery of tests to evaluate mutagenicity, acute, subacute and chronic oral toxicity, carcinogenicity and teratogenicity. Drug residue depletion and metabolism studies are conducted and, based on toxicity data generated in the safety studies, an assessment of withdrawal times can be made. This is obviously an important component of any development program for production animals such as sheep.

Assuming that safety is established and that projected withdrawal periods are appropriate, broader scale clinical testing is initiated to demonstrate target animal safety and efficacy in studies designed to satisfy regulatory agencies. Formulation can play an important role in

determining the behaviour of the active compound and development of a range of formulations may be useful. Manufacturing and quality control define and ensure process and quality standards for both bulk drug and formulated product, stability is assessed, and studies to evaluate environmental safety and handler safety are conducted. The final registration dossier is then filed, leading to the regulatory process which ultimately may result in approval of the product for marketing. The regulatory process varies from country to country and incomplete harmonization of requirements and standards for registration is an issue. As a result, certain studies may be specifically required for certain markets and this can also impact the decision as to whether or not a candidate for a sheep anthelmintic may be brought forward for development.

The research process is complex and only a very small number of active compounds find their way through the entire process. While there are always exceptions, the Animal Health Institute (AHI), a U.S. trade association that represents 80% of the animal health products industry, reports that for every 7,500 compounds identified with activity, only three are submitted for registration and only one is eventually approved for commercial sale, and that this process may take 11 years. The cost of developing a new product may be in the \$30–50 million range, although the fully allocated costs of discovery, development and registration of a new chemical entity on a global scale may exceed \$100 million.

A positive aspect of the antiparasitic class is that many of the actives can be used across species, making the search for new compounds in this category a major target. It is for this reason that animal health companies continue to support programs to discover novel parasitocides, incorporating conventional screening and targeted drug discovery to identify potential candidates. It can therefore be expected that new parasitocides will continue to be identified, but, as stated, it is a rare event when a new chemical entity is discovered and makes it through the full development process. Requirements for a successful anthelmintic candidate for development are stringent, with ivermectin serving as the gold standard against which all others are measured. Isolating and developing a compound to compete with this class constitutes a significant challenge, and would need to be applicable to species other than sheep to justify

the cost of development.

More effective robotic screening methods and better forms of targeted drug discovery can be expected to enhance the process, but identification of new actives which would provide significantly improved safety or efficacy spectra over the current industry standards is a challenging objective.

In the absence of new chemical entities, wider application of existing actives can be further exploited through formulation development, ensuring optimal delivery for specific strategic or tactical programs. In the field of formulation diversification, ivermectin for sheep provides an example of a product formulated as a drench, low-volume drench, an injectable formulation, oral tablet, a combination with a flukicide and, most recently, a controlled release capsule providing 100 days activity. While not all of these products are available in all markets, and all formulations would not necessarily be of use on all properties, the need for flexibility in choosing appropriate dosage forms for specific sets of circumstances can be expected to stimulate continued development of new products.

Financial Considerations

There are a number of issues surrounding the animal health and sheep industry that have an impact on the prospects for development of new sheep parasiticides. Within the industry there has been a trend towards consolidation of animal health companies in recent times. The AHI had nearly 60 member companies twenty years ago. Today it has 27, of which a shrinking number are research based corporations (Anonymous 1996a). In addition, many of those companies involved in active basic research in animal health are relatively small divisions of human health pharmaceutical companies, where veterinary drug discovery groups may compete for resources which could be applied to potentially more rewarding human health programs. However, there is a positive side to this relationship in that there is opportunity to share resources and to capitalize on screening systems and scientific leads, which become available through activities focused on human health products. The majority of animal health products generate relatively little revenue — 87% of products in the U.S. market had sales of less than \$1 million dollars in 1994 and less than 1% had sales of more than \$25 million. Despite this, the

industry in the U.S. spends more than \$400 million annually on R & D. This constitutes 18% of sales, which is a higher ratio than for most industries. Of this, as much as 17% may be spent to defend existing registrations. Despite increasing research expenditure, the number of products approved in the U.S. have declined over time (Anonymous 1994). The net effect is that animal health companies are under relatively high pressure to focus attention on markets which offer the best returns, and the sheep anthelmintic market is relatively small in global terms. For instance, although parasiticide sales constitute approximately 25% of global veterinary pharmaceutical sales, sales of sheep products represent only 7%. In Australia, the major sheep market in the world, anthelmintics make up almost one third of the animal health pharmaceutical market, but Australia represents only 2% of global animal health sales and 6% of global parasiticide sales (Anonymous 1966b). Parasiticide sales in Europe and North America constitute more than 60% of global parasiticide sales and, as a result, future developments in the antiparasitic field are largely driven by requirements for these markets, in which cattle and dogs play major roles.

Sustainable Control Measures

While prospects for introduction of a product from a new action class remain uncertain, it is essential that steps be taken to protect the resource that current anthelmintic compounds represent. This translates into adoption of practices that minimize the rate of selection for resistance, while providing appropriate levels of control. It will become increasingly important to communicate appropriate control strategies effectively to both advisors and producers and technology transfer will therefore continue to become increasingly important (Murrell 1996).

Efforts to encourage use of the full effective dose, rotation strategies, quarantine drenching and testing to establish the status of resistance on various properties will need to be promulgated in addition to promotion of well-designed integrated control programs. As new developments evolve in the field of non-chemo-therapeutic control, it will be important to position these measures appropriately to ensure optimal benefit within control programs. It will be necessary to fully exploit the features of existing products and practical implications of research findings on the disposition of antiparasitic drugs

(Hennessy 1994) may become increasingly important to ensure optimal use of the limited resource which current anthelmintics represent. Formulation diversification may further serve to enhance utility of existing actives.

Computer modelling will expand and provide a useful basis for exploring recommendations for product use and for evaluating the impact of management strategies on parasite populations. However, validation and testing of key assumptions and concepts generated in this manner will be important to ensure validity of conclusions and projections.

The Future

Despite the challenges, it is expected that industry will bring new alternatives to the market for control of parasites in livestock. However, the timing is not predictable and, even when new products are brought forward, the challenge of the future will be to implement effective and rational parasite control programs in an environment of evolving production systems and socio-economic conditions.

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PROSPECTS FOR VACCINES AGAINST GASTROINTESTINAL HELMINTH PARASITES OF RUMINANTS

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ABSTRACT

Vaccines for several of the more important species of ruminant helminth are being developed, spurred on by the increasing frequency of anthelmintic resistant strains of worms. In general the search for protective antigens has consisted of preliminary trials to screen candidate protective fractions, then to purify the protective molecules from the most promising fractions, and, finally, to clone and express the genes which encode them, so that protective recombinant DNA antigens can be synthesised.

Several highly protective antigens have been identified, but so far recombinant versions of such highly protective antigens have been produced only for *Taenia ovis*. Of the gastrointestinal helminths, *Haemonchus* and *Fasciola* are likely to be the first targets for defined antigen vaccines. To date, the most promising antigens are various hidden worm gut proteinases for *Haemonchus* and secreted cathepsin Ls and haemoglobin for *Fasciola*.

Keywords: Ruminant helminths, vaccines, protective antigens, hidden antigens, homologous antigens, natural antigens, proteases, integral gut membrane proteins, oncosphere surface antigens, glutathione-S-transferases, recombinant proteins.

INTRODUCTION

With the notable exception of the one for the bovine lungworm, *Dictyocaulus viviparus*, there are no commercially available vaccines for the control of helminth infections in ruminants. Liver flukes and gastrointestinal nematodes are nearly always controlled by a combination of anthelmintic drugs and pasture management. Although this practice can be extremely effective, it is not possible on certain types of farm and on others it can prevent the best use of the grazing that is available. It is also not sustainable, as the situation is threatened by the advent of strains of

worms resistant to anthelmintics. This is particularly acute and widespread in nematodes of sheep and goats in the southern hemisphere where, for example, there are strains of *Haemonchus contortus* resistant to five chemical groups of anthelmintic (Van Wyk, Malan & Randles, 1997; review by Jackson, 1993). Fortunately for cattle farmers, the situation is not yet so serious, although anthelmintic resistant *Trichostrongylus axei* and *Fasciola hepatica* have been described and in New Zealand a recently isolated strain of *Cooperia* was not susceptible to either oxfendazole or ivermectin (Vermunt, West, and Pomroy, 1995). Furthermore, concerns have been raised about anthelmintic residues both in food and in the environment, where adverse effects on harmless or beneficial fauna have been described.

Unfortunately there is no news of unrelated, completely novel anthelmintic drugs approaching the market place; the latest products are all variants of known chemical groups. Because of this, of the expense involved in developing new compounds and of some promising results with immunisation against other parasites (e.g. the Australian cattle tick, *Boophilus microplus*), some commercial companies are investigating the potential of vaccines against ruminant helminths, usually in collaborative projects with government or university-funded scientists. If successful, control by vaccination could offer an escape from most of the difficulties outlined above for anthelmintics. Using specific examples, the present paper will concentrate on some of the strategies that are being used to achieve this goal, rather than to attempt to provide an up to date list of the various protective antigens that have been discovered for the different helminth species of ruminant helminth.

Vaccine strategies

Early attempts to immunise ruminants against gastrointestinal helminths, either with crude worm homogenate antigen or by ectopic infection (where live worms were administered by an unnatural route as "vaccine", e.g. *Ostertagia* given intraperitoneally), met with little or no success.

The first breakthrough came in the 1960s when it was discovered that infection with *Dictyocaulus* larvae which had been attenuated by irradiation could stimulate a high degree of protection against challenge with intact, non-irradiated infective larvae. This discovery led to the development of "Dictol", the vaccine which is still sold today. Attempts were immediately made to extend the principle to the gastrointestinal helminths, but unfortunately it was found that while the method worked well under experimental conditions for *H. contortus* and *T. colubriformis* in sexually mature sheep, in field infections the protective effect was either too weak or too variable to be a practical proposition particularly in young lambs. Of course, no helminth vaccine can be commercially successful if it is not efficacious in young, highly susceptible ruminants on pasture. For reasons that are still unclear, immunity to *Dictyocaulus* spp. can develop rapidly and effectively in the calf or lamb whereas immunity to the gastrointestinal nematodes is acquired much more slowly.

Much research has been and is still being conducted into the mechanisms of naturally acquired immunity to gastrointestinal helminth infections of sheep and cattle, but it is still not clear what the final effector mechanisms are. Unfortunately the situation seems to be complex involving a combination of local hypersensitivity in addition to cell mediated, antibody and inflammatory responses, and is complicated by the natural unresponsiveness which exists in the young lamb or calf and in the dam around parturition. Only if and when these and the respective antigens which trigger them are finally unravelled, can attempts be made to artificially stimulate them.

The vaccine strategy currently enjoying most success ignores the mechanisms of natural immunity, but attempts to direct high titre antibody responses towards potentially susceptible targets on or secreted by the parasite. In the case of blood feeding species the luminal surface of the intestine has been a particularly rich source of suitable target molecules. Because molecules on the luminal surface of the parasite intestinal cells are not normally recognised by the host during infection, these antigens are classified as "hidden", a feature which raises some unconventional concepts relating to vaccination strategy. However, in the case of *F. hepatica* some success has been achieved using natural antigen as well as a

"homologous" antigen, that is an antigen first shown to be protective in another helminth species. Finally, work with *Taenia ovis* has revealed an example of a protective "natural" antigen. Unlike *Fasciola* and the gastrointestinal nematodes, it is well known that circulating antibody plays a role in naturally acquired immunity to *T. ovis*, a situation which allowed the identification of the first recombinant protective antigen for any helminth of ruminants.

Examples of "hidden", "homologous" and "natural" protective antigens for ruminant helminths are described in more detail below. The general approach for "hidden" and "natural" antigens has been first to screen candidate protective fractions enriched for the parasite target in preliminary protection trials, second to purify the protective components as much as possible and finally to isolate and express the genes which encode these so that a functional recombinant protein can be produced. As the adult stages of the economically important species of ruminant helminth cannot be satisfactorily cultured *in vitro*, it can be relatively difficult and/or expensive to obtain sufficient quantity of worms for the first two of these steps and large numbers of parasite donor animals are required. This partially explains why so much more work has been done with ovine rather than bovine parasites, the exception being adult *Fasciola* which can be obtained relatively cheaply from the abattoir. It is generally assumed that if protective antigens can be identified in a nematode species which infects sheep, it should be relatively straightforward to identify the homologous proteins in the equivalent bovine parasite. Of course, the final step of producing a functional recombinant antigen is crucial to the whole approach, because a helminth vaccine which consists of native worm antigen can never be a commercially viable proposition.

1) Hidden antigens

a) worm gut membrane proteins

The gut membrane approach was first applied successfully to ticks in research which culminated in the launch of the recombinant vaccine against *Boophilus microplus*, the Australian cattle tick (Willadsen, Bird, Cobon & Hungerford 1995). The principle is straightforward. The host is immunised with appropriate gut membrane proteins from a haematophagous parasite and a high titre circulating antibody response is raised.

5 enzymes from plasmids gut
identified so far

When the parasite subsequently feeds on the host, it ingests antibodies which bind to functional proteins on the brush border of its intestinal cells, so that its digestive processes are compromised, leading to starvation, loss of fecundity and weakness. Eventually the parasite becomes detached and, in the case of the gastrointestinal species, is swept out of the gut by peristalsis. This technique is showing great promise as a method for controlling the blood feeder, *Haemonchus contortus*, from which several different gut membrane proteins or protein complexes have been isolated, giving more than 80% reduction in eggs together with greater than 50% protection against worm numbers when tested under experimental conditions (Jasmer, Perryman, Conder, Crow & McGuire 1993; Smith, Munn, Graham, Tavernor & Greenwood 1993; Smith, Smith & Murray 1994; Knox, Smith, Smith, Murray & Redmond 1993). So far, with one exception (Jasmer, Perryman and McGuire 1996), these antigens have been identified as various proteases which, it is presumed, are involved in the digestion of the blood meal (Smith, Graham, Munn, Newton, Knox, Coadwell, McMichael-Phillips, Smith, Smith & Oliver, 1997; Redmond, Knox, Newlands & Smith 1997; Longbottom, Redmond, Russell, Liddell, Smith & Knox 1997). The thrust of current research is to produce these antigens in recombinant form.

A major advantage of the hidden antigen approach is that, because the mechanism of immunity is quite different, it works in situations where natural immunity to *Haemonchus* is weak or ineffective. Thus it has been shown that young lambs (Tavernor, Smith, Langford, Munn & Graham 1992; Smith 1993), goat kids (Jasmer & McGuire 1991) and periparturient ewes (Andrews, Hole, Munn & Rolph 1995) can be successfully immunised and that some protective immunity is even transferred by maternal antibody (Andrews *et al* 1995). On the other hand, the fact that the antigens are hidden means that, unlike conventional vaccines, immunity is not boosted by infection. At first sight this seems a serious disadvantage because it might seem that frequent immunisations would be required for an effective level of protection to be maintained.

However, experimental evidence suggests otherwise. The mechanism of protection in lambs immunised by this method mainly affects late fourth-stage and older worms, whereas

incoming larval stages which are not yet blood feeders are largely unaffected (Smith & Smith 1993). Thus, when immunised lambs are subjected to repeated daily infections of larvae, to mimic the situation in the field, faecal egg counts and adult worm numbers are controlled by the vaccine and the continued presence and activity of the early larval stages stimulates a natural immunity which is capable of replacing the effects of vaccine immunity when this wanes (Smith & Smith 1993). Therefore the hope is that *Haemonchus* can be controlled both by immunising lambs before weaning and ewes during pregnancy to prevent the periparturient rise in egg count.

Another potential advantage of hidden over natural antigens is that, because the host does not respond to them during natural infection, the parasite has not needed to adapt to counteract the host response. Thus hidden antigens are likely to be conserved, both within and probably between genera. Certainly, the protective gut membrane aminopeptidase from *Haemonchus*, often referred to as HII, has been shown to be effective against geographically distant isolates of worms and, on an equally practical note, against anthelmintic resistant strains (Smith & Smith 1993; Newton, Morrish, Martin, Montague & Rolph 1995).

It is not entirely clear whether the gut antigen principle can be successfully employed against species of nematode which are not direct blood feeders. The precise diet of economically important species like *Ostertagia* and *Dictyocaulus* is not known, but they contain host immunoglobulin, presumably ingested with mucus, tissue fluids and/or serous exudates (Murray & Smith 1994). It remains to be determined whether the amounts consumed are adequate for this vaccination strategy to be effective, but there is at least one encouraging result with *Ostertagia circumcincta*. (Smith, Smith, Murray, Liddell & Knox 1993). Incidentally, even though Dictol is effective, because it is live and attenuated, it is cumbersome to manufacture and distribute, so that a vaccine for *D. viviparus* based on a defined antigen would be an attractive alternative.

b) non-gut proteins

Glutathione-S-transferase from *F. hepatica* (described below) is a hidden antigen because it is not recognised serologically by sheep or cattle

infected by this parasite (Sexton, Milner, Panaccio, Waddington, Wijffels, Chandler, Thompson, Wilson, Spithill, Mitchell & Campbell 1990).

2) Homologous antigens

The glutathione-S-transferases (GST) of *F. hepatica* were chosen as candidate vaccine antigens because homologous proteins from *Schistosoma mansoni* and *Schistosoma japonicum* had been shown to be protective in laboratory animal model infections (Mitchell 1988). GSTs are involved in the metabolism of xenobiotics, transport of anionic compounds and the detoxification of lipid peroxides. Sheep and cattle immunised with native GSTs isolated from *F. hepatica* have been protected on average by 49 and 29%, respectively, although the results from individual trials have been quite variable.

It is not clear by what mechanism protection is induced. The simplest possibility is that antiGST antibody neutralises these enzymes, which normally counteract the effects of reactive oxides produced by the inflammatory response of the host.

Even though it has been found that GSTs are unlikely to be useful antigens for *Haemonchus*, there is no doubt that the approach of using DNA or antibody probes to identify protective antigen homologues between parasites will become more and more commonplace and should provide a valuable short cut to the laborious protein fractionation procedures which have mainly been employed to date.

3) Natural antigens

Technically, the most advanced defined antigen vaccine for ruminant helminths, indeed for any helminth parasite, is that for *Taenia ovis*, where 3 distinct highly protective recombinant antigens have been synthesised. All three are oncosphere antigens which are recognised serologically by naturally infected sheep. The first one to be discovered, designated To45W, (Johnson, Harrison, Lightowlers, O'Hoy, Cogle, Dempster, Lawrence, Vinton, Heath & Rickard 1989) has been extensively tested with great success in field trials in New Zealand. Two further antigens known as To8 and To6.17 appear to be just as promising (Harrison, Heath, Dempster, Gauci, Newton, Cameron, Robinson, Lawrence, Lightowlers & Rickard 1995). However, *T. ovis* is

not a human or sheep pathogen. Although harmless, the cystic stage in meat has occasionally been a trade barrier for the export of lamb from New Zealand, as it presents an aesthetic problem to the diner. Therefore, for political and marketing reasons commercialisation of the vaccine has not yet occurred (Rickard, Harrison, Heath & Lightowlers 1995).

Cathepsin L from *F. hepatica* is an example of an enzyme which is recognised by the host immune response following infection, and which can induce high levels of protection when used as a vaccine antigen. Vaccination of sheep with native enzyme reduced *Fasciola* egg production in sheep by 70% (Wijffels, Salvatore, Dosen, Waddington, Wilson, Thompson, Campbell, Sexton, Wicker, Rowen, Friedel & Spithill 1994), whereas one report on a trial in cattle claimed 50% reduction in fluke numbers. This effect was improved to 70% in cattle immunised with cathepsin L combined with fluke haemoglobin, and it was found that almost 100% of the eggs shed by the vaccinated animals failed to embryonate, suggesting that parasite transmission would be enormously reduced (Dalton, McGonigle, Rolph & Andrews 1997).

Interest in this protease was originally aroused when secretions from *Fasciola* were found to degrade immunoglobulin and it was suggested that if the enzyme responsible could be neutralised by antibody, the flukes would become more susceptible to the host response (Chapman & Mitchell 1982). Flukes recovered from cattle vaccinated with cathepsin L were retarded, as might be expected if this hypothesis were true, but the mechanism which inhibited egg embryonation was not clear (Dalton, *et al.* 1997). It has been suggested that the parasite uses a cathepsin protease for egg production and that antibody raised by the secreted protease in the vaccine cross-reacts with this, interfering with egg synthesis (Spithill & Morrison 1995). If this second hypothesis is correct, luck will have played a large part in the discovery of this antigen!

Striking protective effects have recently been reported against *Haemonchus*, using low molecular weight antigens derived from adult worms (Schallig & Van Leeuwen 1997). Although other proteins were present in this antigen fraction, it was enriched for two proteins of 15 and 24kd, which are also present in excretory-secretory products of this parasite and which are

recognised serologically by infected sheep (Schallig, Van Leeuwen & Hendriks, 1994). It will be interesting to see whether these antigens are as effective in the young lamb and periparturient ewe where natural immunity is poor.

How good do worm vaccines have to be?

Conventional wisdom suggests that a worm vaccine would have to approach the efficacy of either anthelmintics or bacterial and viral vaccines to be effective. In the case of the gastrointestinal nematodes of ruminants it is more appropriate to consider a vaccine as an epidemiological tool to maintain low level pasture contamination, rather than a weapon to abolish infection completely. Barnes, Dobson & Barger (1995) used a mathematical model they developed for simulating *Trichostrongylus* populations in grazing sheep, to compare theoretical vaccines of nominal efficacy with conventional control methods based on anthelmintic treatment. They concluded that if the vaccine consisted of a natural antigen, only 60% efficacy in 80% of the flock would bring substantial benefits; and if the vaccine was based on a novel antigen, then protection of 80% of the flock or 80% efficacy would give better control than a conventional control programme.

CONCLUSIONS

The prospects for defined antigen worm vaccines are brighter than ever. Scientifically, the concept has been proven for *T. ovis*. In the case of *Haemonchus* and *Fasciola* it is probably only a matter of time before effective recombinant versions of the known protective native antigens are produced. Hopefully the principles can be extended to other important species. Lets hope it will not be too long before vaccines can be added to the existing methods for controlling helminth infections of grazing ruminants.

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ECOLOGICAL SELECTION FOR ANTHELMINTIC RESISTANCE: RE-EVALUATION OF SHEEP WORM CONTROL PROGRAMS

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ABSTRACT

The occurrence of anthelmintic resistance in sheep nematodes has reached a critical stage in Australia, with resistance to the benzimidazoles, levamisole and their combinations now affecting almost all properties in major sheep areas. The macrocyclic lactone (ML) anthelmintics represent the sole remaining effective group in many situations, but ivermectin resistance has recently been detected in *Ostertagia* on a number of farms in Western Australia.

In contrast to reports from other countries, however, these cases do not appear to have arisen due to an excessive frequency of anthelmintic treatment. In the cases investigated, sheep had usually been dosed with an ML anthelmintic on only one or two occasions per year. Resistance was first detected after as little as 4 years of the sole use of ivermectin.

It appears likely that the rapid selection for resistance to an initially highly-effective anthelmintic has been due to ecological constraints on reinfection at a critical point in the annual infection cycle. Because of the hot and dry summers experienced in inland Western Australia, negligible proportions of nematode infective larvae survive this period. This has been the basis of a widely-used strategic treatment program, which results in minimal worm burdens for many months after a single summer treatment. However, any resistant survivors of these treatments would become the parent generation of future worm populations, and hence the level of resistance could rapidly increase.

Strategies to reduce the rate of selection for resistance are urgently required. Experiments in progress aim to ensure the survival of small numbers of non-selected worms in sheep through summer, to dilute resistant individuals. Approaches being tested include the administering of treatments in late spring, when small numbers of non-selected worm larvae will be acquired by sheep, and the treatment in

summer of only part of the flock.

These treatments options may appear radical to sheep producers, but fortunately, in environments where the heaviest selection for resistance occurs, parasite infection pressure is not usually severe. Conversely, in less extreme environments, where a higher level of over-summer larval survival favours greater parasite activity, and more frequent treatments are needed, the selection pressure is reduced by a dilution effect. It will be essential to define situations where the deliberate retention of a small worm burden does not lead to appreciable production loss, if strategies to permit sustainable nematode control are to be accepted by the sheep industry.

ANTHELMINTIC RESISTANCE IN AUSTRALIA

Anthelmintic resistance in nematode parasites of sheep was first confirmed as a widespread problem in Australia some 20 years ago (Webb, McCully, Clarke, Greentree & Honey 1979), and is now considered to be nearly universal on intensively-managed properties. Resistance chiefly involves the genera *Haemonchus*, *Ostertagia* and *Trichostrongylus*, and the benzimidazole (BZ) and levamisole (LV) anthelmintics (Overend, Phillips, Poulton, & Foster 1994). More recently, resistance to closantel has also been detected in *H. contortus* (Rolfe, Boray, Fitzgibbon, Parsons, Kemsley & Sangster 1990), and to ivermectin in *H. contortus* (Le Jambre 1993) and *Ostertagia* (Swan, Gardner, Besier & Wroth 1994).

The development of resistance to the macrocyclic lactone (ML) anthelmintics usually marks a serious departure from the potential for successful worm control, as by the time this has occurred, resistance is usually advanced in the older anthelmintic classes. New chemical alternatives are not likely to be available to sheep farmers for some years (Soll 1997), and alternative control possibilities are expected to be either some time away or may not reach fruition (Barger 1996). The economic impact of

anthelmintic resistance has been shown to be substantial (Besier, Lyon & McQuade 1995), and will inevitably increase as viable control options decline.

In this critical situation, it is vital that sheep farmers employ measures to delay further increases in anthelmintic resistance. Logically, the factors considered to most contribute to the development of resistance in a particular situation should be identified, and emphasised in advice to producers. Although the single most important factor is undoubtedly the excessive exposure of nematode populations to anthelmintics, the strong selection for resistance applied by a small number of treatments at ecologically-critical times has received less attention. The link between environmental factors and selection pressure is suggested by the rapid development of resistance to the ML anthelmintics on some sheep properties in Western Australia, supported by experimental studies. It is considered that in some environments routinely-used strategic programs pose a serious risk of promoting the development of resistance, and that a revaluation of some strategic approaches is required.

TREATMENT FREQUENCY AND IVERMECTIN RESISTANCE

Measures to reduce the exposure of nematode populations to anthelmintics has long been the central element in recommendations to combat anthelmintic resistance (Prichard, Hall, Kelly, Martin & Donald 1980), and worm control extension campaigns to producers have concentrated chiefly on the need to minimise the number of annual drench treatments (Waller, Dash, Barger, Le Jambre & Plant, 1995; Besier 1997).

The emergence of resistance to the most recently introduced anthelmintic class, the ML group, provides an opportunity to investigate the factors most significant in the development of resistance in different situations. As a relatively recent phenomenon, the history of anthelmintic treatment and sheep management is usually more easily related to resistance status compared to the BZ and LV anthelmintics, to which resistance was often present for some time before detection. Detailed investigations so far involve only ivermectin, the first drug of the group to be available.

Although ivermectin resistance is still relatively rare on a global scale, reports from countries in Europe, South America, Africa, southern Asia and the Pacific Islands (summarised in Waller 1997) indicate that a high frequency of treatment is considered to be responsible in most cases. In South Africa, a number of severe cases of ivermectin resistance have also been attributed to very frequent treatment, although the origin of others involving fewer known treatments was not apparent (van Wyk, Malan, Gerber & Alves 1989). In some reported cases, anthelmintics were administered at intervals close to nematode pre-patent periods, virtually guaranteeing that the only surviving worms are resistant.

However, in Western Australia, resistance to ivermectin was first detected in *Ostertagia* in sheep treated on only 12 to 16 occasions over 4 years (Swan *et al.* 1994). In 3 subsequent confirmed cases for which a detailed case history is available, ivermectin resistance had developed after even fewer annual treatments (Besier 1996). In no cases were either the under-dosing of sheep with ivermectin nor the importation of resistant worms in introduced sheep believed to be relevant. The only common factors apparent are the exclusive use of ivermectin for at least 4 years, and the administration of most treatments in summer as part of a strategic control program.

Although these cases involved *Ostertagia*, this genus is not likely to have a greater genetic propensity for the development of ML than others, as in many situations where ML resistance was found in only *H. contortus*, other genera present would have experienced similar levels of anthelmintic treatment. In fact, initial investigations suggest that ML resistance in *H. contortus* involves a single, dominant gene (Le Jambre 1993), a genetic basis which could hardly be more conducive to the development of resistance.

In the absence of a high frequency of treatment, an explanation for the rapid development of ivermectin resistance in *Ostertagia* was sought in the relationship between the timing of treatment regimens and environmental effects on the free-living stages of nematodes.

ENVIRONMENTAL INFLUENCES AND STRATEGIC TREATMENTS

The potential for interactions between environmental factors and anthelmintic treatment practices to apply selection pressure in favour of resistant worms has been recognised theoretically for some time (Le Jambre 1978; Donald 1983), but interpretations of the importance has varied and there has been little field investigation (Waller 1993).

The rate of development of resistance is influenced by the proportion of the nematode population in *refugia* when anthelmintics are administered, and hence able to escape the selection pressure applied to adult worms (summarised in Martin 1990; Prichard 1990). Surviving resistant worms may be diluted by others developing from the non-selected population, and the mean level of resistance reduced. However, where conditions are hostile to the survival of nematode free-living stages for prolonged periods, or a pasture has been kept free of larval contamination to provide worm-free grazing, there may be comparatively little infection with a non-selected population following treatment. Although sheep treated during this period can remain relatively worm-free for a considerable period, selection for resistance would be expected.

There are indications of this unintended result in observations of field cases where sheep were treated with benzimidazoles and moved to worm free-pastures (Cawthorne & Whitehead 1983; Martin, Anderson & Jarrett 1985). In contrast, Waller, Donald, Dobson, Lacey, Hennessy, Allerton & Prichard (1985) recommended caution in discrediting dose-and-move strategies, as in field experiments in New South Wales they found this practice provided substantial production benefits, and did not select more heavily for resistance than in set-stocked sheep given a similar number of treatments. However, Martin (1989) noted that the low selection pressure in that instance may have been due to sufficient infective larvae remaining on the pasture between sheep moves to dilute resistant survivors of treatments.

Sheep production systems in Mediterranean climatic regions of Australia such as the South West of Western Australia would appear to be particularly vulnerable to selection pressure exerted by environmental effects on nematode larvae. Pastures typically dry off over summer, and few infective larvae of the principal nematode genera of sheep, *Ostertagia* and

Trichostrongylus, survive from spring to the following autumn (Anderson 1983; Young 1983). Recommended sheep worm control programs capitalise on the poor over-summer larval survival, through a "summer drenching" program based on treating sheep in late spring and in mid-summer (Anderson 1972, 1973; Besier & Lyon 1990). A common variant in cereal-growing areas is to treat sheep as they are moved onto dry crop stubbles, on which there is virtually no reinfection with nematode larvae (Wroth 1996). The extreme effectiveness of these strategies suggests the potential for heavy selection pressure for anthelmintic resistance.

FIELD EVIDENCE FROM WESTERN AUSTRALIA

The relationship between the over-summer survival of infective larvae selection and the potential for anthelmintic resistance has been demonstrated in ecological studies and field observations in two areas of different climate in Western Australia. Studies were conducted at Badgingarra, 150 km north of Perth, Western Australia, and in the milder climate of Mount Barker, 350 km south east of Perth.

Larval survival over summer

In both locations, the over-summer survival of infective larvae of *Ostertagia* and *Trichostrongylus* was assessed by depositing worm eggs in sheep dung onto pasture plots twice-monthly from October until March or April for 2 (Mt Barker) or 3 (Badgingarra) years, and assaying the plots for infective larvae at intervals, with final observations when autumn rains had led to vigorous pasture growth. The pastures on the plots were similar to those grazed by sheep in both locations, and are chiefly annual clover and grass species which are dry over summer, and grow for different periods depending on rainfall.

Badgingarra experiences hot and dry summers (Fig. 1), with daily maximum temperatures frequently exceeding 40 °C in summer. The effective pasture growth season lasts for only about 5 months each year. Ecological studies (Table 1) indicated that although infective larvae developed from spring depositions of worm eggs, only minute proportions survived over summer to be present on pasture in autumn (Wroth 1995a). Most depositions of eggs in summer and early autumn failed to yield

Figure 1. Long-term mean monthly maximum and minimum temperatures and rainfall at Badgingarra, 150 km north of Perth in Western Australia

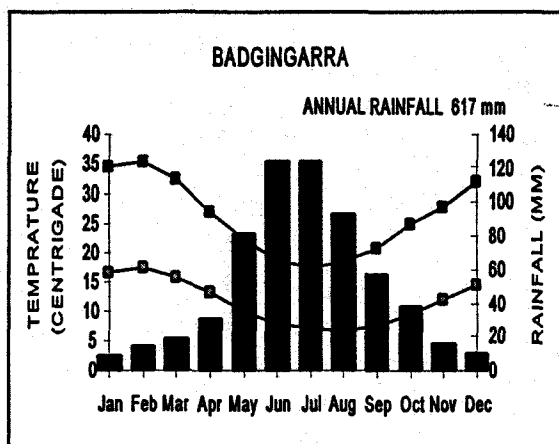
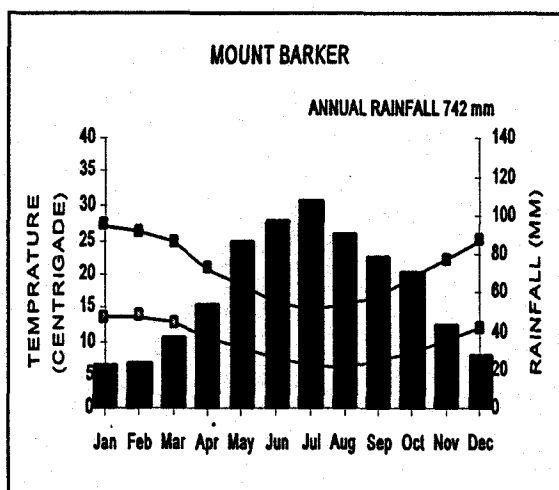


Figure 2. Long-term mean monthly maximum and minimum temperatures and rainfall at Mt Barker, on the south coast of Western Australia



infective larvae at any time.

In contrast, Mt Barker experiences milder summers with more rainfall, and maximum summer temperatures rarely reach 35 °C (Fig. 2). The pasture growth period exceeds 7 months. Small but consistent recoveries were made of infective larvae of *Ostertagia* and *Trichostrongylus*, which developed in spring and survived through summer in sheep faecal pellets (Table 1; Besier & Lyon 1990). Similar observations of over-summer larval survival were made in earlier ecological studies in Victoria (Anderson 1983; Young 1983) at sites also

experiencing milder summer conditions than at Badgingarra.

Observations on worm burdens in grazing sheep in Western Australia confirm that infections are considerably greater in intensity and duration in areas with climates similar to Mt Barker, compared to those with extreme summer conditions such as Badgingarra (B.Besier, unpublished).

Selection for anthelmintic resistance

Evidence for selection for anthelmintic resistance by summer drench treatments at Badgingarra derives from field experiments in that area by Wroth (1992, 1995b), which compared the rate of development of benzimidazole resistance in initially-susceptible *Ostertagia* and *Trichostrongylus* spp in sheep treated with anthelmintics at different frequencies and times of the year. Results of worm egg hatch assays for benzimidazole resistance over 5 years confirmed that both the frequency and timing of treatment were important, with the heaviest selection for resistance in sheep treated monthly. However, high levels of resistance developed in sheep given two drenches in summer, or four treatments in winter, and resistance developed rapidly after even a single treatment in summer. In contrast, resistance developed slowly after two treatments in winter.

Ivermectin resistance and environment

The selective potential of this environment provides an explanation of the geographic distribution of field cases of ML resistance in Western Australia. Of the 7 cases so far confirmed, all have occurred in areas with hot and dry summers similar to those at Badgingarra, (Besier, unpublished). Of 4 cases of ivermectin resistance for which the detailed treatment history is available, most sheep received only one or two drenches each year (Besier 1996).

Although no studies comparable to the Badgingarra experiments have been conducted in the Mt Barker environment, the absence of detected instances of ML resistance, despite a similar level of testing by farmers, suggests a lower level of selection pressure. In these climatic zones, most farmers follow the recommended two-treatment summer drench

Table 1. Recovery of infective larvae in autumn from pasture plots contaminated with *Ostertagia* and *Trichostrongylus* eggs in sheep faeces 2–3 times per month from October to March. Results are mean percentage recoveries for all depositions in each month for 1988/89 & 1989/90 (Mt Barker), and 1991/92, 1992/93 & 1993/94 (Badgingarra)

Month of faecal deposition	Mean percentage larval recovery		
	Mt Barker (2 years)		Badgingarra (3 years)
	<i>Ostertagia</i>	<i>Trichostrongylus</i>	<i>Ost.</i> and <i>Trich.</i> combined
October	0.0376	0.0048	0.0003
November	0.0564	0.0003	0
December	0.0037	0.0016	0
January	0.0014	0.0012	0.0003
February	0.292	1.136	0.0066
March	Over 1%	Over 1%	0.097

program (Besier & Lyon, 1990), and additional winter treatments are common. Compared to drier areas where most sheep receive a single treatment as they are moved onto crop stubbles (B.Besier, unpublished observations), a higher prevalence of resistance would be expected if a high frequency of treatment was the dominant causal factor.

The tendency for lower selection pressures in milder climatic areas is consistent with the paucity of reports of ML resistance levels in other areas within the Australasian region where sheep are managed in a similar manner. Ivermectin resistance has not been detected on commercial properties in the summer rainfall area of Northern New South Wales, where nematode infection levels and treatment frequency are considerably greater than in Western Australia (P.Rolfe, personal communication). In field experiments in the uniform rainfall region of near Sydney, ivermectin resistance was not detected despite up to 8 treatments to sheep per year for 3 years (Waller *et al.* 1985). In New Zealand, where more frequent anthelmintic treatments than in Australia are usual, ML-resistance has not been detected in sheep nematodes (P.McKenna, personal

communication).

DEVELOPING APPROPRIATE CONTROL STRATEGIES

The circumstantial and experimental evidence regarding ML resistance in Western Australia supports a strong relationship between selection pressure and larval survival of *Ostertagia* and *Trichostrongylus*. This provides a basis for matching control strategies to the environment and the major parasites, to help preserve long-term anthelmintic effectiveness as well as to control worm burdens.

The extent and duration of larval survival can be considered an objective index of potential selection pressure. Although ecological data is usually available from only a limited number of locations in most countries, extrapolations may be made from areas of similar climate, and observations of the seasonality and composition of worm burdens are of value. Computer simulation models, when validated for a range of environments and different nematodes, will allow the exploration of a wide range of control options and the estimation of the potential level of selection (Barnes, Dobson & Barger 1995; Leathwick, Vlassoff & Barlow 1995).

In environments where nematode larvae survive on pasture for long periods of the year, the greatest danger for the development of severe anthelmintic resistance usually remains an excessive frequency of treatment. As the greater challenge is usually to effectively control worms in the short term, the number of annual treatments required in control programs may be relatively high. Even though this may not be considered highly selective, however, the informed use of grazing management routines and timely monitoring of worm burdens should be encouraged to help reduce the need for anthelmintics.

In situations of high selection potential, however, both the number and timing of drenches are significant, and revisions of worm control recommendations may be indicated. The unfavourable nature of the pasture environment for the free-living stages of parasites for long periods makes worm control generally easier than in milder climates, and offers considerable scope to develop pasture movement routines to protect susceptible sheep with the minimal use of anthelmintics. However, even with the most

innovative pasture manipulations, effective anthelmintics will be required on some occasions.

New approaches to "summer drenching" programs in Western Australia aim to preserve the beneficial effects of the present strategies, but to avoid the heavy selection pressure associated with these programs in regions of extreme summer conditions.

NEW APPROACHES FOR HIGH-SELECTION ENVIRONMENTS

In winter-rainfall regions, the advantage in favour of resistant worms can be reduced by deliberately aiming to preserve a non-selected population in sheep through summer. Although the concept of encouraging the survival of a worm burden rather than attempting to remove it may appear contradictory to the aims of control programs, any adverse effects are expected to be more than offset by the longer-term benefits of preserving the effectiveness of anthelmintics. It will be important to ensure that only the minimum burden necessary is tolerated, both to avoid adverse effects on sheep and to retain effective strategic control. Fortunately, in populations where anthelmintic resistance is below the level of detection, the number of resistant individuals surviving treatment is very low, and will be effectively diluted by even a relatively small number of non-selected worms.

Two strategies to retain a small non-selected worm burden have been considered feasible in this environment. Firstly, all summer treatments could be avoided, and sheep drenched when the annual plant species of pastures senesce in late spring and infective larvae populations are in rapid decline (Wroth 1996). This treatment is intended to control existing worm burdens, but to allow the subsequent acquisition of small numbers of worms deriving from the less-selective winter and spring periods. However, it is difficult to estimate the size of the worm burden likely to be acquired after drenching, and there is a risk that this may be either too large to be acceptable, or too few to be effective.

A second strategy involves the deliberate withholding of treatment to a proportion of a sheep flock when summer drenches are given, so that a non-selected worm population remains to contaminate pastures and lead to the dilution of resistant worms in all sheep in the flock after

the autumn rains (Barnes *et al.* 1995). While this strategy may require the toleration of some lost production in non-treated sheep, choosing those in the best nutritional condition will reduce any adverse impact on a flock basis. This approach also allows the proportion to be left untreated to be decided objectively on the basis of the mean flock faecal worm egg count before treatment. Computer simulations suggest that this strategy is more reliably effective in a winter-rainfall environment than the former (R.Dobson, unpublished).

Experimental work to examine these strategies in several environments within Western Australia is proceeding, and the extent of loss in sheep production associated with the deliberately-retained worm burdens will be investigated. The results will also provide the basis for further computer simulations, so that strategies and variations appropriate for particular environments can be predicted without the need for extensive local investigations.

CONCLUSIONS

With the inevitable increase in the impact of anthelmintic resistance, control programs must focus on both the effectiveness in worm control, and the longer term preservation of anthelmintic efficiency.

Although the recommendation to reduce the frequency of treatment to the minimum necessary remains central, the role of relatively few treatments at ecologically-critical times should receive greater prominence. The environmental circumstances and associated importance of modifying treatment strategies vary with location, but the principle of using larval ecology as an index of the selection pressure is universal.

However, it is critical that caution is used in developing recommendations which may compromise the effectiveness of worm control in order to reduce the selection pressure. There is a danger that by attenuating the strategic effect, a larger number of treatments may be required—perhaps with the ironical result of both increasing the selection pressure and impairing worm control. Also, revisions to strategic programs will not reduce the value of grazing management and timely monitoring of worm burdens, which themselves can reduce the level of selection.

Novel strategies to reduce the selection pressure are likely to encounter resistance to adoption by some sheep producers, who may be sceptical of programs which may appear to compromise worm control. It will be important that farmers seek professional guidance on programs to minimise selection for resistance, as the strategies under investigation will provide only a basis for individual planning. Periodic monitoring of worm burdens will be required, and perhaps annual assessments to take account of the seasonal conditions and sheep management options. It will also be important to ensure that sheep introduced from properties where low-selection strategies have not been adopted are thoroughly de-wormed on arrival. Unfortunately, as anthelmintic resistance becomes more severe, effective control options become more complex.

Provided that low-selection control strategies appropriate to the environment are implemented, however, individual producers may be able to delay the onset of severe resistance until longer-term solutions such as the breeding of sheep genetically resistant to nematodes begin to take effect.

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